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University of Nebraska-Lincoln, sprajitna@gmail.com

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**EVALUATION AND ANALYSIS OF BEEF CONTAMINATION BY LOW
LEVELS OF AMMONIA**

By

Sely Prajitna

A THESIS

Presented to the Faculty of
the Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Food Science and Technology

Under the Supervision of Professor Randy L. Wehling

Lincoln, NE

April, 2011

EVALUATION AND ANALYSIS OF BEEF CONTAMINATION BY LOW LEVELS OF AMMONIA

Sely Prajitna, M.S.

University of Nebraska, 2011

Advisor: Randy L. Wehling

Leakage of ammonia in food facilities has been known to occur in the past. Ammonia leaks often lead to food contamination in food plants and can cause illness among consumers who accidentally consume the food products contaminated with high levels of ammonia. Therefore, a rapid, simple yet accurate method has to be established for on-site ammonia level screening in food plants to ensure the food is safe. One of the primary objectives in this study was to verify and optimize an ion selective electrode (ISE) method for its accuracy and reproducibility in determining ammonia in meat. Different extraction procedures (blending and vortexing) and different solvents (nanopure water with/without pH adjustment (control, 3.0, 4.0, 5.0, and 6.0) using either HCl/NaOH or HClO₄/NaOH; potassium phosphate buffer (PPB) at different concentrations (0.1M, 0.02M and 0.01M) and pH (5.8, 6.0, 6.2, 6.5, 6.8, 7.0, and 7.2) were tested to improve the performance of the ISE method. Blending spiked meat with pH 6 0.01M potassium phosphate buffer (PPB) as the solvent was shown to give better ammonia recovery and lower coefficients of variation. The recoveries were >90% with coefficients of variation ranging from 3.6 to 14.2% for ammonia concentrations ranging from 10 to 200ppm in spiked meat samples.

This study further investigated the rate of ammonia uptake in fresh and frozen meat samples (2.5x2.5x1 inch) exposed to 200ppm ammonia in N₂ gas at three different

temperatures - ambient (20-25°C), refrigeration (3-5°C), and frozen temperatures (-13°C), for times of 1 to 12 hours. A selected packaging film (2.4 mil Cryovac type B6620) was also tested for its permeability to ammonia gas during 12 hours exposure at 3-5°C. The rate of ammonia uptake in fresh meat was 58.4 ± 7.1 ppm per hour for 20-25°C during 6 hours time exposure and 56.4 ± 5.8 ppm per hour for 3-5°C during 9 hours time exposure. A sign of saturation was observed after exposing fresh meat for more than 9 hours at 3-5°C. Frozen meat had a slower ammonia uptake rate which resulted in an ammonia concentration approximately 6 times lower than the ammonia level in fresh meat samples after 12 hours exposure. Moreover, the 2.4 mil Cryovac type B6620 packaging film provided a good barrier to ammonia gas. The ammonia concentrations (ppm) between both non-exposed (control) and vacuum-packed meat samples exposed to 200 ppm NH_3 gas at 3-5°C showed no significant differences ($P < 0.05$).

Selected removal methods (1 to 2 hours air flushing, 2 hours vacuum treatment, and 2% acetic acid rinsing) for lowering ammonia levels in contaminated meats were also investigated in this study. All of the removal methods showed minimal ammonia reduction (<10%) in contaminated meat samples exposed to 200 ppm NH_3 gas for 4 hours at 3-5°C.

DEDICATION

I would like to dedicate this thesis to my parents, Sjöfian and Rasmi, for their support, encouragement and unconditional love in my whole life. I would also want to extend this dedication to my brother, Anton, and my sister, July, for their support and love.

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INTRODUCTION

Leakage of ammonia in food facilities can lead to food contamination at a level that changes the quality of the food and makes it not safe for human consumption. Due to no established standard method available for safety screening, many ammonia contaminated food products were distributed and sold to consumers without knowing that the foods were contaminated. Although there are many methods available for detecting and quantifying ammonia in different substances, most of them are expensive, require extensive operational training, meticulous extraction and purification procedures, an extensive pre-incubation period, or use of expensive materials or hazardous chemicals. A rapid, simple yet accurate method is preferred for a fast paced on-site ammonia level screening in food plants to ensure that food is free from ammonia contamination.

Even though the rates of ammonia uptake in meat exposed to different ammonia levels, temperatures, and exposure times have been studied in the past, there is still some missing important information in those studies. Thus, this study was conducted to fill in the missing information. Moreover, due to a lack of studies investigating the effectiveness of techniques to reduce or remove ammonia in contaminated meat, most of the ammonia contaminated food products were considered as non-safe food for consumers and were destroyed.

Therefore, the objectives of this study were: 1) To verify and optimize an ion selective electrode method for accuracy and reproducibility in determining ammonia in meat. 2) To investigate the rate of ammonia uptake by fresh and frozen beef exposed to 200ppm ammonia gas at selected times and temperatures. 3) To investigate selected

techniques (air flushing, vacuum treatment, organic acid rinsing) for lowering ammonia levels in contaminated meat.

Chapter I

Literature Review

Chemical and Physical Properties of Ammonia

An ammonia molecule has a molecular weight of 17.031g/mol, and it appears in the shape of a trigonal pyramid consisting of four charge clouds (three single bonds of H atoms and one lone pair) surrounding the N atom in the center (McMurry and Fay, 2001). Due to the presence of the lone pair, the angle of H-N-H is diminished to 107° and is less than the ideal 109.5° tetrahedral angle (McMurry and Fay, 2001). An ammonia molecule itself has a dipole moment of 1.47D (McMurry and Fay, 2001). This net molecular polarity/dipole moment of ammonia is created by the electron lone pair of nitrogen, and the nitrogen atom is more electronegative relative to hydrogen atoms (McMurry and Fay, 2001). The partial negative charge on the electronegative nitrogen atom can also interact with the partial positive charge on neighboring a hydrogen atom to form hydrogen bond (McMurry and Fay, 2001). The ability of ammonia to form hydrogen bonds affects the boiling point and solubility of ammonia (McMurry and Fay, 2001). Some other properties of ammonia are shown in Table 1.

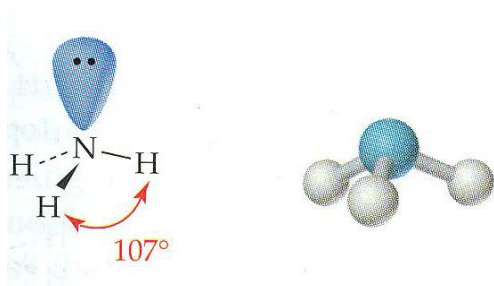


Figure 1. Three-Dimensional structure of Ammonia molecule (McMurry and Fay, 2001)

A few distinct characteristics of ammonia are it is a colorless gas with a distinct pungent smell and can be easily detected by the human nose at a concentration of 35ppm

or above (Al-Sahal, 2003). Ammonia in the form of gas is known as anhydrous ammonia. Ammonia is quite soluble in water. When it comes in contact with water, it forms ammonium ion (NH_4^+) and becomes odorless and non-gaseous (Dworkin et al., 2004; ATSDR, 2004). The ionic form of ammonia in water is known as aqueous ammonia. The reaction between ammonia and water molecules is reversible which is shown in Equation 1a or 1b. (McMurry and Fay, 2001; Dworkin et al., 2004). In water solution, not all ammonia molecules will react with water to form ammonium ion; only some ammonia molecules interact with water molecules via intermolecular interaction in the water solution (McMurry and Fay, 2001). The ability of ammonia to solubilize in water is possibly due to its molecular polarity and hydrogen bond formation. The unshared pair of electrons in an ammonia molecule binds to any free H^+ atoms in water solution, resulting in ammonium ion and hydroxide ion which is shown in Equation 1a (McMurry and Fay, 2001). As more ammonia dissolves in water, production of hydroxide ions (OH^-) in the water solution will increase; thus they will increase the pH of water to alkaline pH depending on the concentration of ammonia gas dissolved in water solution.



Or can be written as follows:



Property	Value	Reference
Molecular weight	17.03	LeBlanc et al. 1978
Color	Colorless	LeBlanc et al. 1978
Odor	Sharp, intensely irritating	Sax and Lewis 1987
Odor threshold:		
Air	25ppm (18mg/m ³)	Amoore and Hautala 1983
	48ppm (34 mg/m ³)	Leonardos et al. 1969
	53ppm (38mg/m ³)	Budavari et al. 1996
Water	1.5ppm	Amoore and Hautala 1983
Physical state	Gas at room temperature	LeBlanc et al. 1978
Melting point	-77.7°C	LeBlanc et al. 1978
Boiling point	-33.35°C	LeBlanc et al. 1978
Density:		
Gas	0.7710 g/L	Weast et al 1988
Liquid	0.6818 g/L (-33.35°C, 1 atm)	Windholz 1983
Vapor density	0.5967 (air = 1)	Windholz 1983
Vapor pressure:		
Anhydrous NH ₃	10.2atm (25°C)	Daubert and Danner 1989
Aqueous NH ₃ (28%)	2.9atm (25°C)	Daubert and Danner 1989
pKa	9.25 (25°C)	Lide 1998
Solubility:		
Water		
at 0°C	47% (w/w)	Budavari et al. 1996
at 15°C	38% (w/w)	Budavari et al. 1996
at 20°C	34% (w/w)	Budavari et al. 1996
at 25°C	31% (w/w)	Budavari et al. 1996
at 30°C	28% (w/w)	Budavari et al. 1996
at 50°C	18% (w/w)	Budavari et al. 1996
Organic solvent(s):		
at 0°C	20% (w/w) in absolute ethanol	Budavari et al. 1996
at 25°C	10% (w/w) in absolute ethanol	Budavari et al. 1996
	16% (w/w) in methanol	Budavari et al. 1996
	Soluble in chloroform and ether	Budavari et al. 1996
Flammability limits in air	16-25%	LeBlanc et al. 1978

Table 1. Chemical and physical properties of ammonia (Adapted from ATSDR, 2004)

Because ammonia gas is quite soluble in water, any food products with high water content can easily be contaminated with ammonia gas. Solubility of ammonia in water can be affected by temperature and pH. Since ammonia has a boiling point of -33.35°C , the solubility of ammonia in water will decrease as the temperature of the water solution containing ammonia increases above its boiling point. In Table 1, it shows that as the temperature increases by increments of 20°C at a constant pressure of 101kPa, the ammonia solubility in water decreases by approximately 50%. According to Thermo Scientific ISE's user guide, at room temperature, the rate of ammonia escaping from a moderately stirred 100mL basic solution is about 50% within a six-hour time period (Thermoscientific, 2007). An increase in temperature will result in increased kinetic energy of ammonia molecules to a sufficient level to break free from the water solution and escape as gas (McMurry and Fay, 2001). Thus, ammonia gas is highly soluble in water at low temperature. Ammonia gas is also known to be more soluble in ice or water glaze than in water (CSIRO, 2002; Berry, 2009; Hagyard et al., 1993); thus, frozen meat products take up and accumulate ammonia more easily on their surface during exposure to ammonia gas. However, the diffusion rate of ammonia from surface into frozen raw meat is much slower than in unfrozen raw meat (CSIRO, 2002; Hagyard et al., 1993).

Moreover, pH can affect the solubility of ammonia gas in water. Ammonia gas has higher solubility in acidic water than in alkaline water. Based on Figure 2, at low pH (≤ 6), all the ammonia stays in solution in the form of ammonium ions. Thus, low pH food products are more susceptible to ammonia gas contamination (Arnold, 1993). As the pH increases, ammonium ion is converted into ammonia and escapes from water solution as

a gas. Figure 2 shows that aqueous ammonia completely volatilizes at $\text{pH} \geq 12$. Thus, solubility of ammonia in solution is reduced at alkaline pH.

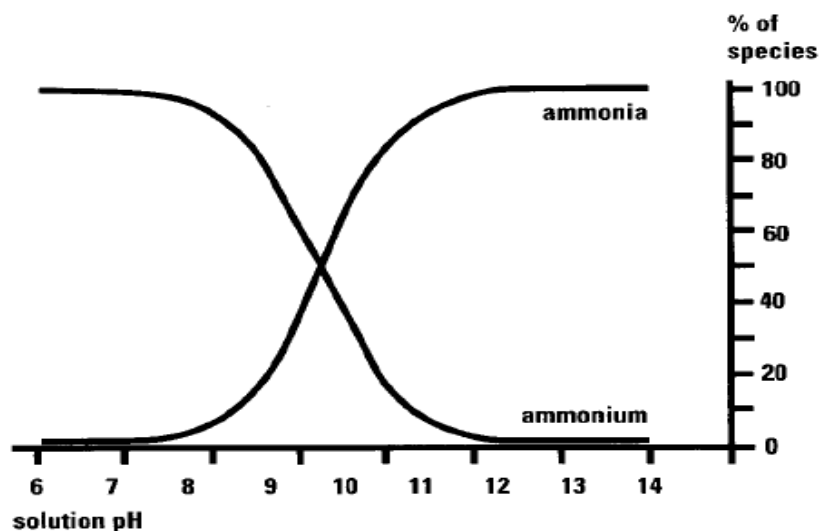


Figure 2. Percentage of ammonia and ammonium ion in solution at different pH (Adapted from Thermoscientific, 2007)

Ammonia is flammable when it reaches a volume of 16% to 25% in the air (Arnold, 1993). It is also categorized as a corrosive type of chemical (Arnold, 1993; HSEES-Oregon); therefore, it is considered as a toxic gas because it can cause harmful effects on people exposed to high concentrations of ammonia. Common effects which have been reported after exposure to ammonia are respiratory, eye, and skin irritation or burning, gastrointestinal problems, headache, nausea, shortness of breath and vomiting (HSEES-Washington; HSEES-NY).

Ammonia Release Incidents in the Food Industry

Accidental release of ammonia gas in food facilities has been reported in the past years. It is an uncommon incident, but it does occur mainly due to equipment failure and human error (HSEES-Iowa). A total of 240 releases of ammonia occurred in Oregon from

1993-2007, and 24.6% of those incidences happened in food related industries (HSEES-Oregon). New York State, from 1993 to 1998, reported about 31.8% of accidental releases of ammonia occurred in the food/beverage industries and groceries/retail. Moreover, there were 378 accidental releases of ammonia gas reported in Washington State from 1993-2001. From the total of 378 cases, 36% of the accidents occurred in non-durable goods manufacturing mainly in dairy facilities, preserved fruit and vegetable manufacturers, and producers of miscellaneous food products (HSEES-Washington). Other ammonia release incidents have been reported in North Carolina. There were a total of 107 accidental releases of ammonia reported during 1993-1998 but only approximately 28% of the total incidents were related to food industries such as meat, dairy, and beverage (HSEES-NC). Minnesota also reported a total of 459 ammonia gas releases during the years of 1995-2005; however, the State's report did not specify where they occurred. It only reported that 36% of the total incidents happened in refrigeration systems, manufacturing and commercial operations in which food manufacturing can be classified into those categories. In addition, based on the Iowa State Hazardous Substances Emergency Events Surveillance (HSEES) report in 2001-2002, about 12% of the total of 219 ammonia releases occurred in the food industry, and they were mainly in meat and poultry processing plants, dairy and ice cream plants, and cold storage warehouses. Moreover, the most recent accidental release of ammonia in 2010 occurring in the food industry was at the Coors Brewing plant in Golden, CO (Gathright, 2010). From all this evidence, it is proven that accidental release of ammonia gas in food facilities does occur.

Due to the accidental release of ammonia gas in food facilities, there are possibilities that food products can be contaminated with ammonia during the accident. The accidental release of ammonia in 2010 led to destruction of food products at a frozen chicken plant in Theodora, AL (Bloomberg Businessweek, 2010). Moreover, according to just-food.com, in 2006 a recall of frozen cooked winter squash, which was contaminated with ammonia, was made by Birds Eye Foods after receiving complaints from consumers (Just-food.com, 2006). Another case reported in the past was frozen beef burritos. In 2001, after the company, Camino Real Foods, received complaints from consumers, they recalled about 40,300 pounds of burritos which were suspected of being contaminated with ammonia (Process Cooling, 2001). In addition, a few cases of ammonia contamination in food products have been reported to cause illness amongst consumers. In 1985, there was an incident of milk contaminated with ammonia ranging from 530ppm to 1,524ppm, causing 20 elementary schoolchildren to become ill within an hour (CDC, 1986). Another case of food contaminated with ammonia was also reported in 2002 in Illinois. About 157 students and teachers became ill after the consumption of cooked chicken tenders (Dworkin et al., 2004). The cooked chicken tenders were contaminated with ammonia ranging from 880ppm to 1,076ppm, and about 552ppm to 2,468ppm in the uncooked frozen chicken tenders. Symptoms reported mostly occurred within one hour (Dworkin et al., 2004), which was similar to the previously reported milk incident. Moreover, according to a CDC report, there have been outbreaks of ammonia poisoning due to ammonia contamination in foods which have not been well documented in the past.

Ammonia toxicity, health implications and excretion

Over exposure to ammonia gas can be hazardous to human health and sometimes even deadly. Accidental releases of ammonia at work areas have caused casualties and injuries among employees in the past. On June 20, 2009 in Robeson County N.C., human error led to the rupture of an ammonia pipe during maintenance causing one death and injury of many employees at a poultry processing plant (Berry, 2009). According to Iowa State HSEES, the major causes of ammonia leaking in most incidences are equipment failure and human error. Exposure of mild to moderate ammonia for a short period of time has been reported to cause many injuries such as, respiratory tract, eye, and skin irritations, gastrointestinal problems, headache, dizziness, and shortness of breath (CDC 1986; HSEES-Washington; HSEES-NY; HSEES-Oregon). Respiratory irritation, eye irritation, and gastrointestinal problems are the most frequent injuries reported after exposure to ammonia. Table 2 provides a summary of symptoms showed after exposure to different levels of ammonia.

Due to the water soluble characteristic of ammonia, it can easily enter the human body by dissolving in skin, mucous membranes, and eyes (ATSDR, 2004). Most of the ammonia gas being inhaled will quickly dissolve into the mucus of the nasal passages forming ammonium ions and be swallowed or dissolved and carried by the blood (Ryer-Powder, 1991). Inhalation of 25ppm of ammonia gas would result in increasing blood ammonia levels by 10% (Ryer-Powder 1991; The Fertilizer Institute). Having an alkaline characteristic, inhalation of ammonia will cause a disturbance of pH in the body, leading to occurrence of all sorts of discomfort or symptoms (Ryer-Powder 1991). Prolonged exposure to high level ammonia can cause a corrosive effect and necrosis of biological

tissue (ATSDR, 2004; Ryer-Powder 1991). As ammonia converts into ammonium ion inside the body, it becomes a highly ionized weak base which can destroy cell tissues by saponifying cell membrane lipids, extracting water from cells, and initiating inflammatory response (ATSDR, 2004). Acute exposure to ammonia is associated with immediate death due to damaged epithelia, edema, secretions, and reactive smooth muscle contraction leading to obstruction of airway (ATSDR, 2004; Issley and Lang, 2009). Survivors from acute ammonia exposure may die later in several days or weeks from infection of damaged epithelial airway (ATSDR, 2004).

The effects of inhalation of different levels of ammonia gas for long times have been studied in the past using animals. Inhalation of ammonia for long periods of time do cause various health problems, for instance, decrease in growth, loss of weight, inhibition of brain functionality, nasal passages and lung damage, hyperemia, etc. (Ryer-Powder, 1991). A study has been done to observe the effects of ammonia gas in poultry houses. Aziz and Barnes (2010) observed reductions in body weight of 17% and 20% when the chickens were raised in an environment containing 50ppm and 75ppm of ammonia gas for 7 weeks, respectively. Accumulation of ammonia gas in poultry houses can also cause eye damage and respiratory diseases. It damaged the mucous membranes of the respiratory system, which made the chickens becomes susceptible to bacterial infection, especially *E. coli* (Aziz and Barnes, 2010). Moreover, exposing rats to 9ppm of ammonia for at least 35hr/week for 4 months showed lower immune system response against bacterial infection (Ryer-Powder, 1991). Due to the toxicity of ammonia gas causing harmful effects on human health, the Occupational Safety and Health Administration (OSHA) has set up exposure levels of ammonia for people working in environments

where there is a possibility of ammonia exposure. The limitation of ammonia inhalation in the workplace is 25ppm for an 8-hour time-weighted average and a short-term (15minutes) exposure limit of 35ppm of ammonia gas (ATSDR, 2004). Based on NIOSH recommendations, the limitation of exposure to 50ppm ammonia gas at the workplace is 5minutes (ATSDR, 2004).

Concentration / Time	Effect
10,000 ppm	Promptly lethal
5,000 – 10,000 ppm	Rapidly fatal
700 – 1700 ppm	Incapacitation from tearing of the eyes and coughing
500 ppm for 30 minutes	Upper respiratory tract irritation, tearing of the eyes
134 ppm for 5 minutes	Tearing of the eyes, eye irritation, nasal irritation, throat irritation, chest irritation
140 ppm for 2 hours	Severe irritation, need to leave exposure area
100 ppm for 2 hours	Nuisance eye and throat irritation
50 – 80 ppm for 2 hours	Perceptible eye and throat irritation
20 – 50 ppm	Mild discomfort, depending on whether an individual is accustomed to smelling ammonia

Table 2. The effects of different ammonia levels on humans without protective clothing (From the Fertilizer institute)

Consumption of food contaminated with ammonia can also cause illness to consumers ranging from mild to severe symptoms and even death if higher concentrations of ammonia, such as 5,000ppm to 10,000ppm are ingested (Dworkin et al, 2004). In past incidents, the levels of ammonia in contaminated food which caused illness, ranged from approximately 500ppm to 1500ppm. During the milk incident, about 20 children became ill after drinking milk contaminated with ammonia ranging from 530ppm to 1,524ppm (CDC 1986). As for the chicken tender incident, the investigator

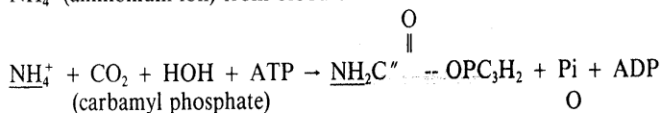
reported that cooked chicken tenders contaminated with 880ppm and 1,076ppm ammonia caused students and teachers to become ill (Dworkin et al, 2004). Chemical foodborne illness does not require activation of the immune system, organism reproduction or toxin production to cause illness; therefore, the symptoms onset time after exposure to ammonia is rapid following consumption of contaminated food products, and it had been reported that the majority of the symptoms occur within an hour after consumption (CDC 1986; Dworkin et al., 2004). Major symptoms observed to occur after consumption of high level ammonia contaminated food products are stomachache, headache, nausea, and vomiting (Dworkin et al., 2004).

Accidentally ingesting certain amounts of ammonia (i.e. household ammonium salts) has led to death in the past. A 57-year-old man was found dead from drinking dilute ammonium hydroxide (2.4% ammonium ion) (Klendshoj and Rejent 1966). Another 69-year-old woman died from acute respiratory distress syndrome and renal failure several days later after ingesting an unknown amount of lemon ammonia (3% ammonium ion) (Klein et al., 1985). Many studies have tested different levels of ammonium in many animals through oral exposure, and different animals showed different effects. A certain level of ammonium is considered a lethal dose for one animal but may not be for other animals (ATSDR, 2004). There was no exact ammonia level threshold established for contaminated food at which consumers started to develop symptoms after consumption of exposed food products. Also, no regulation has been set up to establish the ammonia level in contaminated food products that will result in rejection as not fit for human consumption.

Most ammonia being swallowed or inhaled in the body will be transferred to the liver to be metabolized and excreted out from the body (Ryer-Powder, 1991). Two important metabolism pathways that are important in the human body are the urea cycle and glutamine pathway (Ryer-Powder, 1991). Body organs that are responsible for ammonia excretion and detoxification are the liver and kidney; and, skeletal muscle also plays an important role to detoxify excess ammonia in the human body (Ryer-Powder, 1991). Table 3 briefly shows the conversion and excretion of ammonia by liver and skeletal muscle. In the liver, ammonium ion in the blood will be converted into urea through the urea cycle and then transferred into the kidneys and excreted out as urine (Ryer-Powder, 1991). In skeletal muscle tissue, ammonium ion will be converted into glutamine to be transported into the kidneys (Ryer-Powder, 1991). Glutamine in the kidneys is converted back into ammonium ion and excreted out in urine (Ryer-Powder 1991). Larger amounts of ammonia entering the human body will be excreted out in urine by the kidney (ATSDR, 2004). Ammonia in the body can also be excreted out through the gastrointestinal tract as feces and exhaled out through the respiratory tract, but only a minimal amount of ammonia is excreted through those routes (ATSDR, 2004).

REMOVAL OF AMMONIA VIA THE UREA CYCLE IN THE LIVER

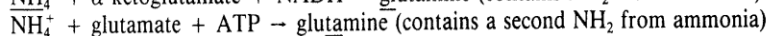
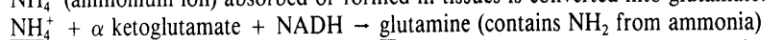
NH_4^+ (ammonium ion) from blood enters the liver where it is transformed into carbamyl phosphate



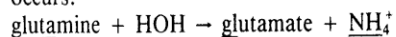
Carbamyl phosphate enters urea cycle and forms urea ($\text{NH}_2\text{C}(=\text{O})\text{NH}_2$) where it is transported via circulation to the kidneys and excreted in the urine

REMOVAL OF AMMONIA FROM OTHER BODY TISSUES

NH_4^+ (ammonium ion) absorbed or formed in tissues is converted into glutamate.



Glutamine as the carrier for ammonia enters circulation and is transported to the kidney where the following reaction occurs:



where the NH_4^+ ammonium ion is excreted in the urine.

Table 3. Excretion of ammonia in human body (Ryer-Powder, 1991)

Sources of Ammonia

Ammonia is a naturally existing chemical which can be found in the atmosphere, soil and water. It is produced through natural breakdown of organic matter (i.e. carcasses, dead plants etc.), animal or human excreta (i.e. manure and urine) or by volcanic eruptions (ATSDR, 2004). However, some ammonia in the environment can be a byproduct formed from manmade processes such as fuel combustion, sewage treatment plants, fertilizer, etc. (ASHRAE). The atmosphere contains an average of 0.3 to 6 parts per billion (ppb) of ammonia, but the level of ammonia will vary/differ depending on the geography, altitude, season, and manmade activities (The Fertilizer Institute). Typically, people who live in larger cities will be exposed to higher levels of ammonia in the air compared to people living in rural areas (The Fertilizer Institute; Ryer-Powder, 1991). Even drinking water in urban areas contains higher levels of ammonia compared to drinking water in rural areas (The Fertilizer Institute; Ryer-Powder, 1991).

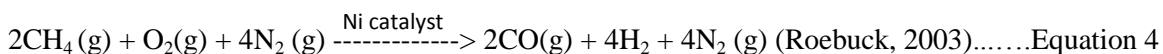
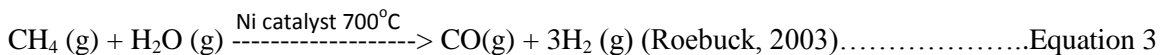
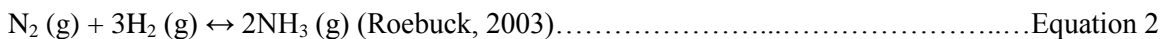
Ammonia produced naturally as wastes by animals and the human body is from breakdown of proteins and amino acids in the liver, skeletal muscle, kidney and stomach; in the intestine, it is also an end product produced as urea and other nitrogenous compounds are broken down by microorganisms (Ryer-Powder, 1991). The human body produces approximately 17g/day depending on the external exposure of the human (The Fertilizer Institute; Ryer-Powder, 1991). External exposure includes breathing air, dietary intake of high protein food products and food additives, drinking water, and smoking cigarettes (The Fertilizer Institute; Ryer-Powder, 1991).

Production of Ammonia

Besides being produced naturally, ammonia has been produced by manmade processes because of its usefulness in many areas of industry. The production of ammonia in the United States has declined in the past years due to increase of natural gas costs and decrease in demands which led to closure of several production plants (ATSDR, 2004). There was a reduction of approximately 39% in annual commercial ammonia production in 2001 (ATSDR, 2004), but then it increased about 13% in 2002 (Kramer, 2003). In 2001, there were 2,338 facilities that produced ammonia in the United States (ATSDR, 2004). Louisiana, Oklahoma and Texas were the three major producing states that contributed to over 50% of total ammonia production in the US in 2000, 2001 and 2002 (ATSDR, 2004).

Ammonia is produced by a process known as the Haber process. This process was developed by Fritz Haber who won a Nobel prize for chemistry in 1918 (Saunders, 2004). In the Haber process, the raw materials of making ammonia are natural gas, air and water

which produce nitrogen and hydrogen (Roebuck, 2003). Thus, a combination of hydrogen (H₂) and nitrogen (N₂) gases under the right conditions yield the end product of ammonia gas. The process of ammonia synthesis requires high pressure, desired temperature, a catalyst and the right ratio mixture of nitrogen and hydrogen gases (Saunders, 2004; Roebuck, 2003). A catalyst is needed in ammonia synthesis to help reduce the activation energy needed to break N₂ and H₂ bonds (Saunders, 2004; Roebuck, 2003). This process yields a gas mixture containing only N₂, H₂ and about 10-20% ammonia as its end products (Roebuck, 2003). As the reactor is cooled, ammonia is condensed to a liquid form and is removed. The unreacted gases, N₂ and H₂, are then recycled back into the process (Saunders, 2004; Roebuck, 2003).



Uses of Ammonia

Ammonia is considered an essential element which contributes to the nutritional needs of living plants, animals, microorganisms and humans. Natural ammonia is converted by microorganisms to nitrate through the nitrification process; the nitrate is further used by plants for protein synthesis (Mensinga et al, 2003). In the human body, it is important for DNA, RNA and protein synthesis (ATSDR, 2004; The Fertilizer Institute; Mensinga et al, 2003). It is converted into glutamine, and the nitrogen molecules being released from glutamine are used in protein synthesis in tissue cells (Mensinga et al, 2003). Ammonia is also important in maintaining acid-base balance in mammals' bodies (ATSDR, 2004). Besides being crucial to living organisms, ammonia is

also an important chemical used in many industries to produce fertilizer, metals, textiles, pulp and paper, refined petroleum, glue, plastic etc. (The Fertilizer Institute; Ryer-Powder, 1991). More than 80% of ammonia produced in the US is used to make fertilizers (Ryer-Powder, 1991). It is also used in production of other chemicals which can be found in cleaning products and in food products. Ammonia in food products is added in the form of ammonium salts to be used as stabilizers, leavening agents and other food additives (Ryer-Powder, 1991). Table 4 shows the maximum allowable levels of ammonium salts that can be added to processed foods which do not pose risks to human health. Besides addition as food ingredients, ammonia has been used as a refrigerant to cool, freeze, and store raw and finished food products in food facilities.

Type of Ammonium Salts	Processed Foods	Maximum Allowable Levels
Ammonium bicarbonate	Baked goods, grain, snack foods, and reconstituted vegetables	0.04-3.2%
Ammonium carbonate	Baked goods, gelatines, and puddings	2.0%
Ammonium chloride	Baked goods	0.001%
	Relishes and condiments	0.8%
Ammonium hydroxide	Baked goods, cheeses, gelatines, and puddings	0.6-0.8%
Monobasic ammonium phosphate	Baked goods	0.01%
Dibasic ammonium phosphate	Baked goods	1.1%
	Non-alcoholic beverages	0.003%
	Condiments and relishes	0.012%

Table 4. Maximum allowable levels of ammonium salts in processed foods (ATSDR, 2004)

Ammonia as Refrigerant

Ammonia is widely used as a refrigerant in refrigeration systems. According to the United Nations Environment Programme (UNEP) assessment in 1995, sixty percent of the refrigerant used in cold storage and food processing applications is ammonia. Due to its advantages as a refrigerant, many food industries, such as meat and seafood facilities; dairy and ice cream facilities; wineries and breweries; fruit and vegetable facilities; and soft drink facilities, have used ammonia type refrigerant in their refrigeration systems for storage, processing, and distribution of raw and finished products (Berry, 2009; HSEES-Oregon; Teamsters; Fairchild, 1995). It has been used to chill meat, seafood, poultry, perishable fruits and vegetables; to freeze ice cream; and, to cool dairy products, beverages etc. Some of the advantages of ammonia type refrigerant are it does not contribute to ozone depletion and global warming. It transforms into fertilizer when it is released into the atmosphere, and it becomes a base when it comes in contact with water (Fairchild, 1995). Ammonia is also biodegradable and inexpensive (Arnold, 1993). It only costs approximately \$0.35 US per pound (Fairchild, 1995); on the other hand, other types of refrigerants, such as hydrocarbon-type refrigerants, cost 6-12 times more per unit volume than liquid ammonia (Lorentzen, 1988). Moreover, due to its unique chemical and physical characteristics, ammonia refrigerant offers a wide range of temperatures, and it has excellent heat transferring properties compared to other type of refrigerants; therefore, ammonia refrigerant has been used widely for refrigeration applications.

Inhibition of Microorganism Growth

Ammonia, in the form of ammonium, has been used in food products for preservation. However, there were some arguments about the safety of the use of ammonia in food products for preservation. There was a study done showing that it could sustain the growth and toxin production of fungi in corn. At low concentrations, 0.5, 1.0, and 1.5% of ammonium hydroxide, the production of aflatoxin by *Aspergillus flavus* was completely inhibited (Thanaboripat et al, 1992). It was able to inhibit bacteria and nematode growth in soil and during corn storage; and, controlled mold on citrus fruits, etc. It has also been reported to be able to preserve fish, dehydrated potato pulp, broken eggs and meat.

In meat products, low concentrations of ammonium hydroxide have proven to be able to reduce bacterial counts in ground goat meat. The effect of ammonium hydroxide on microbial reduction was due to the toxicity of low levels of ammonium hydroxide to the bacteria. At 0.134M, ammonium hydroxide was able to reduce the bacteria count by a hundred-fold in eleven grams of ground goat meat; and at a higher concentration of 0.402M, the reduction was a thousand-fold (Gupta et al 1988). Gupta et al (1988) concluded that addition of low concentrations of ammonium hydroxide would slow down spoilage, and give better shelf life without any apparent color or flavor changes in the ground goat meat. Jensen et al (2009) stated that anhydrous ammonia was able to inhibit the growth of *Salmonella* Enteritidis and *Escherichia coli* O157:H7. In another study done by Liu and Hsieh (1969), they found that ammonium salts have the ability to undermine the ability of many bacteria to produce protease enzymes. Moreover, uses of

low concentrations of ammonium hydroxide in food products may not cause any side effects on human health; ammonium salts are recognized as safe food additives (Gupta et al, 1988).

Meat

Meat naturally contains ammonia but the ammonia level in meat is safe for consumption. Different types of meat, such as seafood, beef, chicken, pork etc., contain varying ammonia concentrations. Hijaz et al. (2007) showed that the background level of ammonia present in beef (ground chuck, eye round, and top loin) without ammonia contamination ranges from 95.6 ppm to 139.3ppm. The ammonia background in uncontaminated meat is due to biodegradation (Parris et al., 1983; Pivarnik et al., 1998) and enzymatic (Pivarnik et al, 1998) processes as the meat ages and spoils over a period of time during storage. Due to its native presence in meat, ammonia has been used as an indicator of meat products' freshness. Total volatile bases, including ammonia and trimethylamine (TMA), is known to be a reliable indicator of fish quality and spoilage (Parris et al, 1983; Ellis et al., 2000)

External exposure of meat to ammonia is unsafe for human consumption, and also can affect the quality of meat products greatly. Some of the meat qualities affected by external ammonia contamination are color (Shaw et al., 1992; Al-Sahal, 2003), flavor (Hagyard, 1993; CSIRO, 2002), pH (Anil, 1971; Al-Sahal, 1995; Al-Sahal 2003), odor (CSIRO, 2002), water holding capacity (Anil, 1971; Al-Sahal, 1995; Al-Sahal 2003), and tenderness (Anil, 1971).

Color

Meat products, especially beef and pork, exposed to ammonia have significant changes in the intensity of the meat color. Beef exposed to ammonia will have a darker color compared to the untreated beef (Al-Sahal, 2003). Al-Sahal (2003) observed a decrease in lightness (L value) and yellowness (b value) in the exposed beef. After cooking, Al-Sahal (2003) observed an increase in redness (a value) in the exposed beef compared to the unexposed. The increase in redness (a value) in cooked beef caused it to appear pinkish, which is an unusual color for cooked beef (Al-Sahal, 2003). A study done by Shaw et al (1992) noted a persistent pink to red color observed in cooked contaminated pork after cooking it to 80°C. It is believed that ammonia might cause the formation of other pigments in the pork which led to this persistent pink in the cooked meat (Shaw et al, 1992). This causes problems that lead to consumer complaints about the meat quality and affect the marketability of meat (Shaw et al, 1992).

Odor and flavor

Moreover, a high level of ammonia contamination in meat products can affect both odor and flavor of the product. The pungent smell can be detected immediately after the exposure of meat to ammonia (CSIRO, 2002). After a certain period of time, the pungent smell of ammonia will not be detected due to ammonia interacting with the moisture or liquid in the exposed food, resulting in conversion from ammonia gas to ammonium ion, which is odorless. Even in the frozen state, ammonia can interact with ice crystals on the surface of exposed food products and become odorless. In this case, it is believed that ammonia contaminated meat can be identified by tasting (CSIRO, 2002).

When meat is exposed to high ammonia levels for a long time, there is a strong ammonia smell noticeable in the meat after cooking (Hagyard et al, 1993). One study also noted that food contaminated with ammonia tends to rapidly develop a rancid flavor (Hagyard et al, 1993). Hagyard et al (1993) stated that a longer exposure time (32 minutes) of lamb's loin to a high concentration of ammonia would hasten the development of rancid flavor over time.

pH

Another effect caused by ammonia contamination of meat is that it can increase the pH of exposed meat (CSIRO, 2002; C. Hagyard et al, 1993). Ammonia gas increases the pH of meat possibly by associating and reacting with the water's H^+ ions in meat giving OH^- ions. Accumulation of OH^- increases the pH of the meat. According to Al-Sahal (1995), exposing beef, pork, and chicken meat to high levels of ammonia (5,000, 10,000, 25,000, and 50,000ppm) for 3, 6, 12, 24, and 48 hours increased the contaminated meat's pH significantly compared to the unexposed meat. There was an increase of 1 pH unit in the beef and pork at 25,000ppm ammonia gas exposure, whereas chicken meat had an increase of 1 pH unit when it was exposed to 50,000ppm (Al-Sahal, 1995). An increase of food pH by 1 or more unit compared to the control or normal food is an indicator of ammonia contamination in the food product (CSIRO, 2002), and it was believed that the meat had been exposed to a high concentration of ammonia for a relatively long period (>120 minutes) (CSIRO, 2002). Since different meats have different buffering capacities, pH may not be a useful factor to indicate ammonia contamination in meat. Sometimes contaminated meat would develop rancid flavor and

contain a high level of ammonia even with only 0.5 unit of pH increase (CSIRO, 2002; Hagyard et al, 1993; Kassem, 1965).

A significant increase in pH was also observed by Al-Sahal (2003) when beef was treated with levels of ammonia gas (500, 1,000, 2,500, and 5,000ppm) for short periods of time (5, 10, and 20 minutes) at room temperature. The beef showed an increase of 1 or more pH unit compared to the control when exposed to $\geq 1,000$ ppm. Both ammonia gas concentration and time exposure have a positive correlation with the pH of the meat. As the ammonia gas and time exposure increase, the pH of the meat also increases (Al-Sahal, 2003). The pH of the meat was higher generally on the outer most layer because, according to Anil (1971), a 0.6cm thickness of meat's outer most layer contained the highest level of ammonia.

Water holding capacity and tenderness

Proteins are the main sources that are responsible for binding water in meat. The ability of proteins to bind water is dependent on the number of reactive groups and their availability to bind water (Forrest et al, 1975); and those are affected by the pH of the meat. pH affects the net charges on the protein's reactive groups. When pH is close to the isoelectric point of the protein, there is a reduction of reactive groups and water holding capacity in meat. In Figure 3, it shows that meat has the lowest water binding capacity at a pH range of 4.9 to 5.3. Having pH below or above that range, the meat will have higher water binding capacity due to more reactive groups in meat. Since ammonia contamination is able to increase the pH of the meat, and according to Al-Sahal's (2003) results, the ammonia contaminated meat has a pH >6 ; thus, the water holding capacity in

the meat is also increased. According to Al-Sahal (2003), an increase in water holding capacity was observed when treating beef with an ammonia level of 1,000ppm for at least 10 minutes. Exposing beef to 2,500 or 5,000ppm for 5, 10, and 20min improved the water holding capacity significantly (Al-Sahal, 2003). Anil (1971) also observed an increase in water holding capacity in frozen beef after exposing it to ammonia for 72hours at -18°C or -4°C . Interestingly, the water holding capacity is less in the first 0.6cm layer of the meat compared to the second 0.6cm layer, even though the first layer pH is much higher than the second layer.

Since tenderness of meat is associated with water holding capacity, changes in water holding capacity will affect the meat firmness, structure and texture. Meat containing high amounts of bound water will have a firm and rigid structure and also a dry or sticky texture. On the other hand, muscle that has less bound water will have a soft and loose structure and also a wet or grainy texture. Based on the study done by Anil (1977), they found that meat exposed to ammonia had an increase in tenderness, and the first 0.6cm layer was more tender than the other layers of the meat.

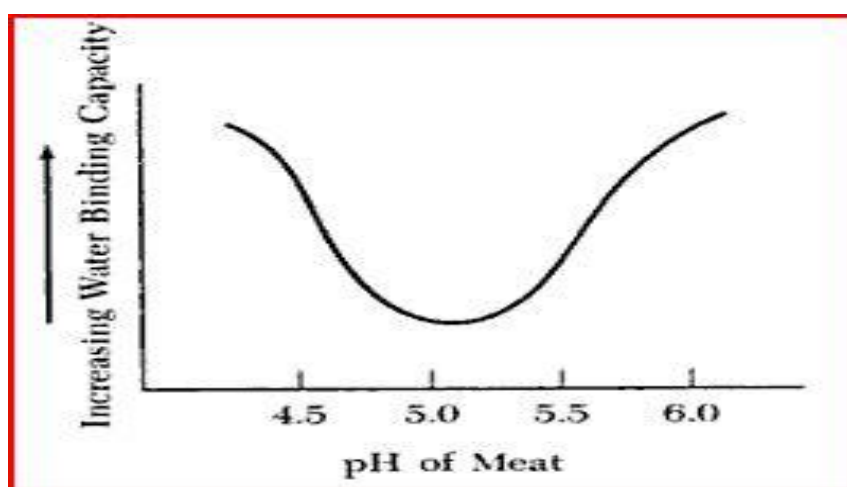


Figure 3. The effect of pH on water holding capacity in meat (Wisner-Pedersen, 1971)

Packaging

Today, the packaging of raw fresh beef has evolved from the concept of 'boxed beef' developed by the French in order to prolong the shelf life of frozen meat for military use (Tomioka, 1990). The beef carcass is cut into primal and subprimal parts to be vacuumed into a low oxygen permeability plastic bag and then placed into a corrugated box for distribution or storage (Tomioka, 1990; Robertson, 2005). The primal and subprimal cuts may then be further fabricated and re-packed for retail to consumers (Tomioka, 1990; Robertson, 2005). The boxed beef packaging concept helps reduce refrigerated space requirements for both storage and transportation and gives ease of handling; however, the main functions of the meat packaging are to contain the meat, and to help maintain and improve the meat quality and safety during storage and distribution. Other functions of the packaging are to protect meat products from physical damage and to improve the attractiveness of meat product displays (Taylor, 1960).

Packaging maintains the quality of meat products by preventing weight loss during storage (Walsh and Kerry, 2005). Weight loss in meat occurs due to water loss from the meat surface through evaporation or sublimation during handling, frozen storage, and distribution; and water loss from meat will lead to texture and color changes in meat (Taylor, 1960). Oxygen/air exposure to fresh meat can also change meat quality through promoting enzymatic activity, bacteria growth, and lipid oxidation. Enzymes in meat systems will still be active even after the animal is converted into a carcass. They continue to consume oxygen and deteriorate meat. The common bacteria causing deterioration in meat products are aerobic bacteria, especially *Pseudomonas spp.*, which require oxygen to grow (Taylor, 1960). In the presence of oxygen, *Pseudomonas spp.* are

able to grow at 0°C in 10days and at 5°C in 5days (Taylor, 1960). Moreover, meat exposure to oxygen will cause lipid oxidation to occur and deteriorate the meat during storage (Walsh and Kerry, 2005). Oxygen exposure also affects meat color, especially in beef (Taylor, 1960). It will become a bright red color when oxygen contacts its surface. Excessive exposure of beef to oxygen for several days will result in a brown color in the meat, which is an oxidized form of myoglobin known as metmyoglobin. This is an unfavorable color for meat, and is perceived as spoiled or not fresh by consumers (Bell, 2001). Controlling the amount of oxygen to which meat is exposed will give the desired meat color and slow down the spoilage or deterioration processes caused by enzymatic activity, bacteria, or lipid oxidation. The role of packaging is to prolong the meat shelf-life by delaying those degradation and deterioration processes by limiting oxygen permeation through the packaging material or altering the atmospheric condition inside the package (Bell, 2001; Taylor, 1960). Packaging also acts as a barrier to protect meat products from external contamination such as dust (Tomioka, 1990), bacteria (Tomioka, 1990), accidental chemical spills, gases, odors, water etc (Bell, 2001).

Packaging methods

Packaging for meat products has evolved throughout the years in order to maintain and improve meat quality and safety during storage and distribution. Nowadays, two types of meat packaging methods are used commonly in the meat industry; they are vacuum packaging and modified atmospheric packaging (MAP).

Vacuum Packaging

Vacuum packaging is the most common packaging method used in the meat industry to wrap carcasses for chilled storage and to transport larger cuts of raw fresh meat from slaughter plants to retail (Bell, 2001). According to Eilert (2005), the use of vacuum packaging methods increased about 3% from 2002 to 2004. Carcasses at the slaughter house are cut into primal and subprimal cuts to be vacuum packed in flexible packaging; meat packed by this method will be further cut, fabricated, and packed in polystyrene (PS) foam or polyvinyl chloride (PVC) trays overwrapped by an oxygen permeable film at the retail location. Some cuts will be sold without further processing (Robertson, 2005). Vacuum packaging improves meat shelf-life, safety, and quality through prevention of moisture loss and removal of oxygen from around the meat (Robertson, 2005). It is able to extend chilled meat shelf-life by up to several weeks (Taylor, 1960; Robertson, 2005). This packaging method also helps to improve meat tenderness and avoids excessive moisture loss while carcasses are hung in a freezer (Taylor, 1960). Packaging materials used for this packaging method must have low permeability to moisture, oxygen and other gases, and be able to resist bone puncture (Robertson, 2005; Seideman and Durland 1983). According to Taylor, there are three basic types of vacuum packaging – cryovac, chamber, and thermoforming. Each type of vacuum packaging uses different packaging materials. Cryovac vacuum packaging is also known as the shrink bag method (Robertson, 2005), and the packaging material used is made from a co-extrusion of three polymeric films of ethylene vinyl acetate/polyvinylidene chloride/ethylene vinyl acetate (EVA/PVDC/EVA) (Robertson, 2005; Taylor, 1960). A cryovac vacuum bag has a tough, clear, heat shrink-ability, and

good oxygen barrier properties; it is commonly used to pack larger primal cuts (Robertson, 2005). A chamber vacuum packaging method is also known as a non shrinkable bag method, and it is commonly used to pack bacon and other processed foods. The films used are nylon or polyester as the outer layer with laminated low density polyethylene (LDPE), surlyn or EVA as the inner layer (Taylor, 1960). The outer layer polymer film gives a good oxygen barrier and provides strength to the bag; the inner layer polymer film, on the other hand, is a good moisture barrier and provides a heat-sealable bag (Taylor, 1960). The thermoforming vacuum packaging method consists of a tray and a flexible plastic film heat-sealed over the tray (Taylor, 1960). This method is commonly used to pack smaller pieces and processed meats (Taylor, 1960). The thermoforming tray is a composite of nylon, polyester or PVC coated with PVDC, and the overwrapping film is made of polyethylene (PE), EVA or surlyn (Taylor, 1960).

Modified atmospheric packaging

On the other hand, a modified atmosphere packaging method is most commonly used for retail packaging in which the raw fresh meat cuts are packed into individual units and are ready to be sold to consumers. This method improves meat shelf-life, safety, and quality by altering the gaseous atmosphere properties surrounding, or in the head space above, the packed beef (Robertson, 2005). However, the main purpose of MAP is to improve the coloration of raw fresh meat and still able to extend the shelf-life of meat by flushing the package with different gases or combinations of gases. Fresh raw meat shipped from slaughter house to retail is usually vacuum packed; and, vacuum packed meat usually appears purple in color due to the lack of oxygen exposure; thus, the MAP

method will help transform the raw fresh meat color, especially beef, from purple to red. Common gases used for the MAP method are O₂, CO₂, and N₂.

Methods for Ammonia Analysis

Several methods have been developed in the past to detect and quantify ammonia, but only a few methods have been developed primarily to detect ammonia in a food product. Methods that have been used in the past to analyze ammonia in different substances include ammonia selective electrode (ASE), chromatography, distillation, enzymatic, salicylate, Nessler's method, pH measurement, sensory test panel, spectrophotometry, and indophenols. Even though there were many methods developed to determine the level of ammonia in different substances, there was no specific procedure established as a standard method to assess food products contaminated with ammonia during accidental release of refrigerant in food processing facilities. It has been suggested that ammonia contaminated foods be tested by a combination of methods including sensory evaluation (Anon, 1981; CSIRO, 2002; Goodfellow et al, 1978), pH method (Anon, 1981; CSIRO, 2002; Goodfellow et al, 1978), AOAC's ammoniacal nitrogen method (Anon, 1981; Goodfellow et al, 1978), and microbiological testing (Goodfellow et al, 1978) to determine whether the foods are safe and fit for human consumption (Anon, 1981). As mentioned in the previous paragraph, the pH value cannot be a reliable test for ammonia analysis in contaminated food because different food products have different buffering capacities (Goodfellow et al, 1978). The sensory evaluation test, on the other hand, has to be conducted by trained panelists in order to detect the small changes in qualities (i.e. aroma and flavor) of food contaminated with ammonia (CSIRO, 2002). Sometimes, the sensory test might not be a reliable test because it is based on

subjectivity (Ellis et al, 2000). Finally, the ammoniacal nitrogen assay may not be reliable as well, because a study conducted by Al-Sahal et al (1998) concluded that the ammoniacal nitrogen content was not affected by different ammonia concentrations in tested food samples. A combination of all the tests would help determine which food products are safe and fit for human consumption, but would be time consuming and expensive, especially for sensory evaluation.

Ion Selective electrode

In comparing different methods of ammonia detection based on previous studies, the ammonia selective electrode (ASE) method is believed to be the most rapid, simple, and inexpensive method, and uses fewer toxic reagents (Hijaz et al., 2007; Pivarnik and Thiam, 1998). It also does not require extensive training to operate (Pivarnik and Thiam, 1998). Thus, it can be efficiently applied in the food industry for in plant ammonia detection and quantification. Unlike enzymatic, Nessler's, spectrophotometric, and indophenol methods, ASE was not affected by either color or turbidity of substances or solutions being measured (Leduy and Samson, 1982; Manca et al., 1988). The ASE method also did not need meticulous extraction and purification procedures, an extensive pre-incubation period, or use of expensive materials or hazardous chemicals that are required in other methods.

Principle of ASE

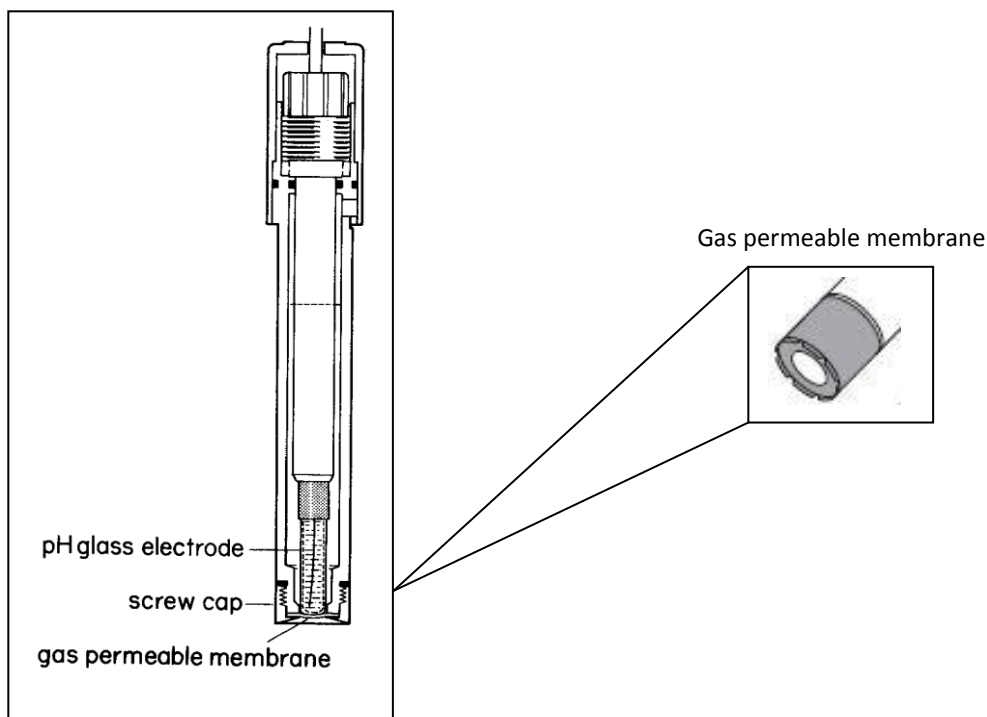


Figure 4. Typical ion selective probe (Evans, 1987)

The concept of the ASE is similar to that of a pH electrode, which is based on the potentiometric principle. It responds to the activity of ammonia dissolved in the internal solution. The internal solution of the electrode is separated from the sample/test solution by a hydrophobic membrane made of polytetrafluoroethylene (PTFE). Changing the pH of the sample solution to alkaline pH allows ammonia to migrate through the membrane into the filling solution inside the electrode. Ammonia reacts with the filling solution reversibly as shown by Equation 1b (Thomas and Booth 1973). The measurement of the ammonia concentration in a sample is generated after the partial pressure of the analyte inside and outside the membrane reaches equilibrium (Evans, 1987). The activity between the ammonia in the internal solution and the thin layer of the glass electrode leads to the potential difference which gives a reading on the meter in millivolts (Thomas

and Booth 1973; Thermo Fisher Scientific, 2007). When the both inner and outer electrode partial pressures equilibrate, the equilibrium constant can be written as follows:

$$K = a(\text{NH}_4^+)/[a(\text{NH}_3) a(\text{H}^+)] \text{ (Evans, 1987)} \dots\dots\dots \text{Equation 5}$$

K is the equilibrium constant. And, because the NH_4^+ activity of the NH_4Cl in the internal filling solution is relatively higher than the activity of NH_4^+ produced by the NH_3 that diffuses through the membrane, the $a(\text{NH}_4^+)$ in Equation 5 is negligible and can be considered as a constant (Evans, 1987). Equation 5 can thus be rearranged and written as follows:

$$a(\text{H}^+) = a(\text{NH}_4^+)/[K a(\text{NH}_3)] \text{ (Evans, 1987)} \dots\dots\dots \text{Equation 6}$$

$$a(\text{H}^+) = \{1/a(\text{NH}_3)\} \text{ (Evans, 1987)} \dots\dots\dots \text{Equation 7}$$

Since the internal electrode consists of a glass membrane and an Ag, AgCl reference electrode similar to a pH electrode, and responds to pH changes in the internal filling solution, the $a(\text{H}^+)$ concentration is proportional to the ammonia concentration (Evans, 1987). Thus, the potential response of the electrode to ammonia can be described as Nernstian (Evans, 1987; Thomas and Booth 1973; Thermo Fisher Scientific, 2007).

$$E(\text{cell}) = E^* + 0.0591 \log a(\text{H}^+) \text{ (Evans, 1987)} \dots\dots\dots \text{Equation 8}$$

$$E(\text{cell}) = E^* + 0.0591 \log \{1/a(\text{NH}_3)\} \text{ (Evans, 1987)} \dots\dots\dots \text{Equation 9}$$

$$E(\text{cell}) = E^* - 0.0591 \log a(\text{NH}_3) \text{ (Evans, 1987)} \dots\dots\dots \text{Equation 10}$$

The partial pressure of ammonia in a sample is proportional to the concentration in the sample (Thomas and Booth 1973; ThermoScientific, 2007). The concentration of ammonia can be determined by using a calibration plot obtained from set of standard ammonia solutions. At low pH, ammonia will remain in solution as ammonium ion. In order to measure ammonia in extracted samples, the pH of the solution must be adjusted to alkaline pH, where the ammonia becomes a gas and diffuses through the hydrophobic

membrane of the electrode. Moreover, a study by Manca et al (1988) concluded that the presence of metal ions such as Mn, Fe, Zn, and Cu in a sample solution will not complex with ammonia and undermine its ability to diffuse through the membrane of the electrode; thus, they will not affect the recovery of ammonia in solution when measured using an ASE. During the alkalization process, which involves adding an ionic strength solvent, most metal ions will form hydroxide precipitates which prevent ammonia-metal complexes (Manca et al, 1988).

Applications of ASE

ASE has been studied widely for use in many areas. It has been used to analyze the level of ammonia in wastewater (Bowman et al, 1986), liquid piggery wastes (Manca et al, 1988), and boiler feed-water (Mertens et al, 1973). It is also used in the medical area to determine ammonia concentration in blood for renal function tests (Georges, 1979; Proelss and Wright, 1973). Other studies have tried to use ASE to analyze ammonia in foods, such as wines and musts (McWilliam and Ough, 1974), seafood (Ellis et al, 2000; Pivarnik et al, 2001; Pivarnik et al, 1998) and meat (Hijaz et al, 2007; Parris and Foglia, 1983). Using ASE to determine ammonia in wastewater has been shown to be a reliable, rapid, precise and convenient method (Bowman et al, 1986). It has also been studied extensively, and shown to be a very rapid method for clinical use to determine ammonia levels in blood (Proelss and Wright, 1973). Hijaz et al (2007) used ASE to analyze ammonia in spiked meat extracts, and they concluded that it was a rapid and simple method, with a high ammonia recovery range from 98.3% to 100.3%, and a standard deviation less than 2%. Parris and Foglia (1983) also obtained a high recovery of ammonia in meat, which ranged from 93% to 100% using ASE. The results obtained

by using an ammonia selective electrode method are comparable and correlate well to the results obtained by the indophenol-spectrophotometer method (Hijaz et al, 2007), enzymatic-spectrophotometer method (Parris and Foglia, 1983), trained sensory method (Pivarnik et al, 2001), and ion chromatography method (Proelss and Wright, 1973)

One of the main drawbacks of using ASE to analyze ammonia in contaminated meat is interferences present in the samples which can cause erroneous responses. Many studies have been conducted to test for possible interferences that might affect the reproducibility, detection limit, and recovery of ammonia by the ASE method. The main interferences were believed to be the volatile amines (i.e. methylamines, dimethylamines, trimethylamines, etc.), which can diffuse through the gas permeable membrane of the electrode during the alkalization (Proelss and Wright, 1973; Moses et al, 1978). Furthermore, amino acids, especially glutamine, can contribute to the non-specific ammonia background when measuring with ASE (Proelss and Wright, 1973). Glutamine could be broken by enzymes and acid hydrolysis producing ammonia over time at room temperature; and, it can also be hydrolyzed liberating ammonia through alkalization during ASE analysis (Proelss and Wright, 1973). Thus, both volatile amines and broken down amino groups can cause erroneous responses by the ASE. Another possible drawback using ASE is the presence of hydrophobic components in the sample solution, such as protein and fat, which can change the permeability of the electrode by fouling its membrane (Proelss and Wright, 1973) leading to decreased membrane shelf life and drift in electrode readings. Besides interferences, another factor that might affect electrode readings is temperature (ThermoScientific, 2007). Differences in temperature will shift

electrode response and change the slope; thus, in order to prevent these problems the temperature of standards and samples must be the same (ThermoScientific, 2007).

Ammonia Extraction Procedures for ASE Analysis

Studies have tried different ammonia extraction procedures to extract ammonia from meat for ion selective electrode analysis. Meat is a highly complex medium consisting of fat, protein, vitamins and other minerals which may interfere with analysis of ammonia in meat. Therefore, the extraction procedure should effectively remove interferences and hydrophobic particles, yet be simple, rapid, prolong the membrane life, give reproducible results and high recovery. Based on the collaborative study done by Ellis et al (2000), ammonia was extracted from fish samples by blending comminuted fish in de-ionized water for 2 min, and measured with an ASE immediately without filtering or centrifuging. This extraction procedure required changing the electrode's membrane constantly because the membrane was fouled easily by the hydrophobic particles in the sample solution; thus, it led to slower response times, low slope readings, and drifting readings (Ellis et al, 2000). Parris and Foglia (1983) suggested the use of alcohol to remove protein and extract ammonia from meat; then, the extract containing ammonia was further centrifuged and filtered for ASE analysis. Others used perchloric acid, a strong acid, combined with centrifugation and filtration to remove protein and extract ammonia from meat samples (Hijaz et al, 2007). According to Proelss (1973), Hijaz (2007), and Georges (1979), removal of proteins using perchloric acid during extraction could minimize some of the problems encountered during ASE analysis. Proelss et al (1973) stated that perchloric acid did remove protein effectively from blood samples and inactivated ammonia-producing enzymes, but it was incapable of removing

amino acids and small peptides. There was also a possibility that the precipitated proteins might bind the ammonia in acidified sample solutions resulting in lower recovery (Ellis et al, 2000). Filtration of meat extracts, on the other hand, could also help reduce hydrophobic components in the solution; thus, it maximized the membrane life, decreased response time of the electrode, and prevented drift in electrode readings (Hijaz et al, 2007). However, a filtration step may reduce the recovery of ammonia by about 10% during filtering of the sample solution (Hijaz et al, 2007).

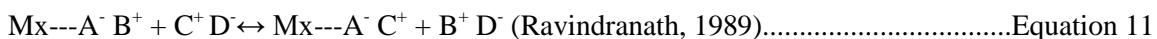
Ion chromatography

Although ion chromatography methods may require tedious extraction procedures for ammonia determination in food products, it eliminates some of the problems encountered by the ion selective electrode. It is able to separate ammonia from other volatile amines in samples, such as methylamines, dimethylamines, trimethylamines, etc. (Proelss and Wright, 1973). Other chromatography methods, such as liquid (Parris, 1984) and gas chromatography (Jenkins et al, 1966) have been used in the past for ammonia determination; however, cation exchange chromatography, combined with conductivity detection avoids the need for chemical reaction of the analyte of interest following extraction.

Principles and Applications of Ion Chromatography

The ion chromatography method used for ammonia analysis is known as cation exchange chromatography, in which the fixed ion in the column is negatively charged, and analyzes ammonia in the form of ammonium ion bearing a positive charge. Negatively charged functional groups in the stationary phases used for ammonium ion

separation included sulfonic acid (Yu et al, 1997), carboxylic (Trifiro et al, 1996), a combination of phosphonic and carboxylic (Trifiro et al, 1996), or a combination of phosphonic, carboxylic and crown ether (Gaucheron and Graet, 2000) as exchange sites. The principle of ion exchange chromatography can be described by the reaction shown in Equation 11, in which the solute $[C^+]$ is separated based on its relative affinity for the fixed ion $[A^-]$ in the column, while competing with the ionic component $[B^+]$ of the mobile phase (Jenke and Pangen, 1987). The mobile phase used usually has a similar charge as the sample ions being analyzed so that they can be eluted from the column.



Where: A^- = fixed cation-exchange group
 B^+ = Mobile phase competing cation
 C^+ = sample cation
 D^- = Co-ion

A common ion chromatography system is shown in the flow diagram in Figure 5. Sometimes the eluent and sample may pass through a guard column before entering the analytical column. This guard column helps to extend the analytical column shelf life through the removal of strongly adsorbed particles found in the sample (Nielsen, 2003).

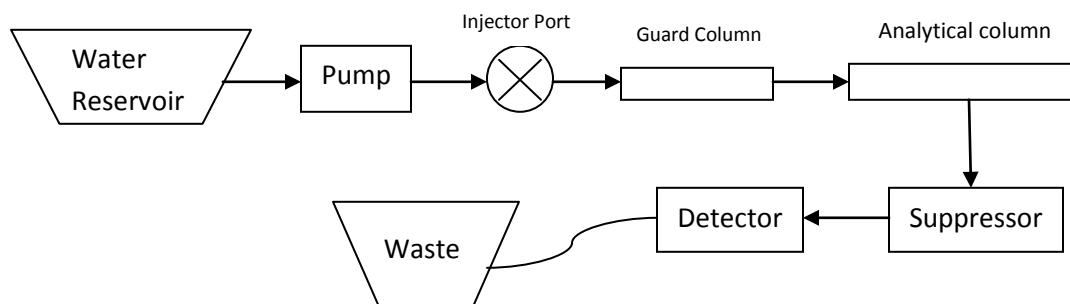


Figure 5. Flow system of cation exchange chromatograph.

The common detector coupled with cation chromatography for detection of ammonium ions is a conductivity detector. Conductivity detectors have been widely used to detect ionic components especially inorganic acids, bases and salts (Beesley and Scott, 1998; Scott, 1995). A simple design for a conductivity detector is shown in Figure 6. It consists of two electrodes in a flow cell which are connected to an amplifier. The sensing cell is built in one arm of a Wheatstone bridge. As ions are passed through the detector cell, they create electrical resistance changes; thus, the amplifier receives those imbalance signals from the electrodes, and it generates an output to a computer or a potentiometric recorder (Beesley and Scott, 1998; Scott, 1995).

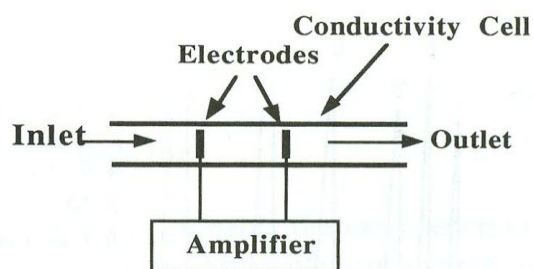


Figure 6. The sensor cell and circuit diagram of an electrical conductivity detector (Beesley and Scott, 1998)

Since the mobile phase used to elute sample ions from the column contains an ionic component and has high ionic strength, it contributes to high background conductivity when the ion chromatography system is coupled with the conductivity detector. Due to this problem, the eluent from analytical column containing the sample is passed through a suppressor before it reaches the detector. The functions of the suppressor are to suppress the mobile phase ions chemically to reduce the background signal produced by the mobile phase ions, and to improve the ionic strength of the sample ions (Ravindranath, 1989). An alternative way to reduce the high background signal

without using a suppressor is to use a column with a very low capacity combined with using a low conductivity mobile phase; thus, the background signal is minimized by that combination. This strategy is known as single column ion chromatography (Jenke and Pangen, 1987). Although the single column strategy gives acceptable resolution, it has a drawback of increased retention time resulting in broader peaks that reduce the sensitivity (Yu et al, 1997)

Due to the problems that might be encountered when using a conductivity detector, a bulk acoustic wave detector (BAW) has been introduced to couple with cation chromatography to determine ammonium in the presence of other ionic components. Yu et al (1997) used a BAW detector along with cation chromatography to determine sodium, potassium and ammonium in human body fluids. The BAW detector performance showed good agreement with conductivity detector and other methods, such as the enzymatic method for ammonium (Yu et al, 1997). By having a coefficient of variation (CV) of 0.8% for 0.50 mmol/L ammonium determination, BAW gives reproducible results (Yu et al, 1997). However, BAW has a higher detection limit, which is 13.9 $\mu\text{mol/L}$, compared to the conductivity detector which has a lower detection limit of 8.6 $\mu\text{mol/L}$ for ammonium (Yu et al, 1997)

Another problem that could be encountered during analysis of ammonium using cation exchange chromatography is nonlinearity of the standard curve at higher concentrations (Bouyoucos, 1977). Due to this problem, a sample containing a concentration above the linear portion of the standard curve has to be diluted until its concentration falls within the linear range. Moreover, sodium in extracts may also pose a problem in ammonia determination by ion chromatography, since the retention time of

sodium and ammonia are close to each other (Huang et al, 2002; Pohl et al, 1999). Quantifying low levels of ammonia in the presence of high levels of sodium may cause the sodium peak to overlap the ammonia peak.

The cation exchange chromatography method has been widely used to determine ammonia levels in many substances, such as biological fluids (Proelss and Wright, 1973), sea water (Huang et al, 2002), dairy products (Gaucheron and Graet, 2000), meat (Yao et al, 1998), and eggs (Yao et al, 1998), and has been found to be more accurate than ASE (Pivarnik et al, 1998).

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Chapter II

Verifying and optimizing an ion selective electrode method for accuracy and reproducibility in determining ammonia in meat

Abstract

Different extraction procedures were tested in this study to optimize ion selective electrode performance in determining ammonia in spiked beef. The extraction procedure plays an important part in acquiring good recovery and reproducibility results for the ion selective electrode method. Blending and vortexing were tested, and our results showed that a blending step gave better reproducibility in extracting ammonia into the solvent. Different solvents with or without pH adjustment were also tested to improve the performance of the ISE method. When extracting spiked meat samples with acidic solvent (i.e. perchloric acid), extra ammonia was extracted out from the tissue sample causing an approximately 50% increase in ammonia level. Comparing the recoveries of solvents with pH 3, 4, 5, and 6, the pH 6 solvent gave the better recoveries. Extracting ammonia using a pH 6 0.01M potassium phosphate buffer as the solvent decreased the variation in ammonia recovery compared to using nano pure water as the solvent. The pH 6 0.01M potassium phosphate buffer gave recoveries >90% with coefficients of variation ranging from 3.6 to 14.2% for ammonia concentrations ranging from 10 to 200ppm in spiked meat samples.

Introduction

Ammonia contamination in food products has occurred in the past. An example of a recent accidental release of ammonia occurred in a poultry plant located in Theodora, AL, in 2010 (Bloomberg Businessweek, 2010; Curran, 2010). Approximately 200 to 300 gallons of ammonia in the refrigeration systems were released from a leaking tank, and led to destruction of some of the chicken products in the plant (Bloomberg Businessweek, 2010; Curran, 2010). Food exposed to high levels of ammonia can affect its quality and threaten food safety. There were two major ammonia related outbreaks among consumers in the past. In 1985, milk contaminated with ammonia caused 20 elementary school children to become ill (CDC, 1986); and, the second incident was in 2002 where students consumed chicken tenders contaminated with ammonia (Dworkin et al., 2004). Although ammonia contamination is known to occur, there is no specific procedure that has been adopted as a standard procedure to assess whether or not a food product is fit for human consumption after being contaminated with ammonia during accidental release of refrigerant.

Measurement of ammonia with an ion selective electrode (ISE) is known to be a reliable, simple, rapid and inexpensive method (Ellis et al., 2000). It is simple and rapid because it has a short analysis time, does not require meticulous extraction and purification procedures or an extensive pre-incubation period. The performance of an ion selective electrode method has been shown to give comparable results to other reliable methods, such as ion-exchange chromatography (Proelss and Wright, 1973), the indophenol assay (Hijaz et al., 2007) and an enzymatic assay (Parris and Foglia, 1983). In addition, it reduces the need for using hazardous chemicals and extensive operator

training, which makes it desirable for an on-site ammonia screening test in the food industry (Ellis et al., 2000). It has been successfully applied to determine ammonia levels in many substances, including wastewater (Bowman et al., 1986), liquid piggery wastes (Manca et al., 1988), boiler feed-water (Mertens et al., 1973), and blood (Georges, 1979; Proelss and Wright, 1973). In applications to food, ISE has been used to determine the ammonia level in wines and musts (McWilliam and Ough, 1974), seafood (Ellis et al., 2000; Pivarnik et al., 2001; Pivarnik et al., 1998) and meat (Hijaz et al., 2007; Parris and Foglia, 1983).

Several extraction procedures for ISE analysis have been proposed. Parris and Foglia (1983) used alcohol as the solvent to extract ammonia from tissue samples. However, this alcoholic extraction procedure only allowed the ISE to measure up to 15 samples prior to deterioration of the electrode membrane (Parris and Foglia, 1983). A collaborative study by Ellis et al (2000) used a water extraction procedure to extract ammonia from fish samples. A few participants in this study encountered problems with the ISE analysis, such as low slope, drifting readings, and slow response time. These problems might be caused by the hydrophobic particles (i.e. protein and fat) in the tissue sample interacting with the electrode membrane, leading to a change in permeability over time and drifting of the electrode potential (Proelss and Wright, 1978). One participant stated that changing to a new membrane could solve the low slope problem (Ellis et al., 2000). Others suggested centrifugation and filtration steps to remove the inferences, which might be the cause of drifting and slow response times (Ellis et al., 2000). Hijaz et al (2007) claimed that the filtration step reduced the recovery of ammonia in tissue samples; thus, acid precipitation with dilute perchloric acid was suggested to remove

proteins from tissue sample extracts. Others have stated that acid precipitation reduced the recovery of ammonia in tissue samples due to the possibility of precipitated proteins binding the added ammonia (Ellis et al., 2000). Because of problems with the extraction procedure and contradictions in studies, the purpose of this study was to verify and optimize an extraction procedure for meat samples analyzed using an ion selective electrode. The results of the ISE were compared to ion chromatography results, which was used as a standard method in this study.

Materials and Methods

Different extraction procedures (blending and vortexing) were tested for better recovery of ammonia and reproducibility of data. Several solvents were also tested for their efficiency in solubilizing and stabilizing ammonia after extraction from meat samples. Solvents tested included nanopure water without pH adjustment; nanopure water with pH adjustment (3.0, 4.0, 5.0, and 6.0) using either HCl/NaOH or HClO₄/NaOH; potassium phosphate buffer (PPB) at different concentrations (0.1M, 0.02M and 0.01M) and pH (5.8, 6.0, 6.2, 6.5, 6.8, 7.0, and 7.2). Different standards (diluted ammonia in solvent and spiked meat extracts) were also tested. Besides using an ion selective electrode (ISE) to determine ammonia levels in samples, cation-exchange chromatography was also used as a standard method for measuring ammonia level in solution. Most of the extraction procedures were adapted from Hijaz et al (2007) with some alteration.

Vortexing Procedure

Extraction

The 96/4 extra lean ground beef purchased from a local grocery store (HyVee, Lincoln, NE) was used in a vortexing procedure to determine the recovery of ammonia. 1.0±0.1g of ground meat in a 15mL disposable screw top centrifuge tube was spiked with 0 (control), 25, 50, 100 or 200 µL of 1000ppm commercial ammonia standard solution (Ricca Chemical Company, Cat No. 615-16) to obtain final concentrations of 0(control), 25, 50, 100 and 200ppm. The spiked 1.0g ground meat was then vortexed (Vortex-Genie manufactured by Scientific Industries Inc., Bohemia, NY) with 10mL volume of solvent (distilled water or 0.3M perchloric acid) for 2 min. The sample solution was allowed to stand for 10 minutes, and then centrifuged at 500x g for 5minutes. The supernatant was filtered through Whatman No. 4 filter paper, and its volume was adjusted to 20mL with deionized water.

Ammonia Determination by Ion Selective Electrode (ISE)

The level of ammonia in the sample was determined using an ammonia ion selective electrode (Orion 9512HPBNWP from Thermo Scientific). The ammonia selective electrode was set up, maintained and stored according to the user guide manual provided by the manufacturer. It was connected to a model 420A Orion pH meter, and the pH meter was set to read in milli-volts (mV).

A series of ammonia standard solutions (1, 10, and 50ppm) was prepared by diluting 1000ppm commercial ammonia standard solution (Ricca Chemical Company) into a solvent (distilled water or 0.15M perchloric acid). Each standard solution was freshly prepared and used to construct a standard curve. A standard curve was

constructed by plotting milli-volts (mV) against the logarithm of ammonia concentration. The levels of ammonia in exposed meat samples were determined by using a regression equation obtained from the standard curve.

For measurement, 20mL each of standard or sample solution was transferred into a 30mL beaker. Measurement of ammonia in the solutions was conducted according to the ISE user guide by adjusting the ionic strength and pH with an ionic strength adjusting (ISA) solution from Thermoscientific. As soon as the electrode was placed into the 20mL of standard solution or filtrate, the pH of the solution was brought to above 11 by addition of 1mL of the pH-adjusting ISA, and mixed evenly by a magnetic stir bar at constant moderate speed. The mV reading was recorded once it was stable. Measurements were made in order starting with standards (low to high), control and then samples. The background ammonia level of an un-spiked meat extract (control) was subtracted from the total amount of ammonia in a spiked meat extract to obtain the actual ammonia added in the spiked meat.

Blending Procedure

Extraction

Eye of round roast beef cuts were purchased from a local grocery store (HyVee, Lincoln, NE). The meat was stored in a freezer as soon as possible after purchase. For determining the level of ammonia in spiked meat, a frozen meat sample was thawed overnight at refrigeration temperature. The meat was ground three times using a bench-top Oster heavy duty meat grinder. 10 ± 0.05 g of ground meat was spiked with 0 (control), 100, 200, 500, 1000, or 2000 μ L of 1000ppm commercial ammonia standard solution (Ricca Chemical Company, Cat No. 615-16) to obtain final concentrations of 0(control),

10, 20, 50, 100 and 200ppm. The spiked 10g ground meat sample was then blended with 90mL of solvent at high speed for 30 seconds using a Waring blender.

Different pHs (5.8, 6.0, 6.2, 6.5, 6.8, 7.0, and 7.2) and concentrations (0.1M, 0.02M and 0.01M) of potassium phosphate buffer (PPB) and nanopure water with pH adjustment (3.0, 4.0, 5.0, and 6.0) using either HCl/NaOH or HClO₄/NaOH, were used as solvent to extract ammonia from exposed meat samples. Each PPB was prepared by combining 1M monobasic and 1M dibasic potassium phosphate, diluting to the specified concentration, and adjusting the pH of the buffer to the desired pH with phosphoric acid (0.1M, 0.02M or 0.01M - according to the concentration of the buffer). The pH was measured by an Orion 9145BN pH electrode connected to an Orion 2-Star Benchtop pH meter. The pH meter was calibrated using pH 4 and pH 7 Orion standardization buffers.

Each blended meat solution was split into two-50mL centrifuge tubes and centrifuged (IEC Centra CL2 centrifuge) at 1000x g for 10 minutes. Most of the ammonia present in the meat is in the form of ammonium ion. Blender processing helps to release the ammonium ion from the meat matrix into the buffer solvent, and the centrifugation step helps to spin down all meat particles, including fat and protein, for ease of filtration. The two-50mL supernatants were combined and filtered through Whatman No.4 filter paper to further remove particles and fat that did not spin down by centrifugation. During the filtration process, a watch glass was placed over the funnel to reduce the rate of ammonium ion escaping as ammonia gas into the surrounding air. The filtrate was adjusted to 100mL with nano pure water. Since meat originally contains a certain amount of ammonia, the 0ppm (control) served as an ammonia background which was subtracted

from the total ammonia level in an exposed meat sample to obtain the level of ammonia contamination in the exposed meat.

Ammonia Determination by Ion Selective Electrode (ISE)

A series of ammonia standard solutions (1, 10, 25, 30, 50, and 100ppm) was prepared by diluting 1000ppm commercial ammonia standard solution (Ricca Chemical Company) into a solvent. A 100mL volume of each standard solution was freshly prepared and used to construct a standard curve.

For measurement, 48mL each of standard or sample solution was transferred into a 100mL beaker. As soon as the electrode was placed into the 48mL of standard solution or filtrate, the pH of the solution was brought to above 11 by addition of 1.6mL of the pH-adjusting ISA, and mixed evenly by a magnetic stir bar at constant moderate speed. The mV reading was recorded once it was stable. Measurements were made in order starting with standards (low to high), control and then samples. The background ammonia level in un-spiked sample (control) was subtracted from the total amount of ammonia in an exposed meat extract to obtain the actual ammonia contamination in the spiked meat.

Ammonia Determination by Cation-Exchange Ion Chromatography

For cation-exchange analysis, 4mL of standards (1, 10 and 25ppm) or sample solutions were filtered through a nylon membrane with 0.45um pore diameter to remove particles from solution before injection. The ammonia concentration in meat samples was determined with a Dionex ICS-3000 chromatograph with dual pumps connected to a conductivity detector. The column used was an IonPac CS 12 analytical column (4x250mm) from Dionex. The IonPac CS12 column is filled with 8µm diameter macroporous particles consisting of ethylvinylbenzene-divinylbenzene copolymer and

carboxylic acid as the functional group of the cation-exchanger. The sample volume of each injection was 25uL. The injected solution passed through an IonPac CG 12 guard column (4x50mm), heated to 30°C, prior to passing through the analytical column. Cations were suppressed with a Dionex CSRS 300 4-mm ion suppressor to reduce background noise. The injections were made with a Dionex AS-100 autosampler held at 4°C. Eluent used for this application was 20mN methanesulfonic acid (MSA) and nanopure water with a flow rate of 1mL/minute. The gradient used for this study was as follows:

Time (min.)	% 20 mM MSA	% Water
0	10	90
27	10	90
27	82	18
30	82	18

There were 10 minutes of equilibration prior to each injection. Samples were tested in duplicate, and runs were analyzed with Chromeleon Client software v 6.80 (Dionex).

Statistical Analysis

The data were analyzed using SAS software (Version 9.2, SAS Inst. Inc., Cary, N.C., U.S.A., 2002). PROC MIXED was used to analyze ammonia recovery differences between methods (ISE and IEC), and solvents, and the ammonia background in meat extracted by water and perchloric acid. LSMEANS was further used to compare treatments for differences.

Result and Discussion

Hijaz et al (2007) and Proelss and Wright (1973) had tested using perchloric acid (HClO_4) for ion selective electrode analysis. Perchloric acid (HClO_4) was used in the extraction procedure to precipitate protein in blood and tissue samples to minimize problems encountered by the ion selective electrode, such as, drifting electrode potential and long response times (Hijaz et al., 2007; Proelss and Wright 1973). Removal of protein from the extract also helped to improve the life of the ion selective electrode membrane and allowed more than 100 samples per membrane (Hijaz et al, 2007). Another study had used alcoholic solvent (i.e. methanol) to remove protein from tissue samples, but using alcoholic extraction only allowed the electrode to measure up to 15 samples before the membrane deteriorated (Parris and Foglia, 1983). Hijaz et al (2007) reported %recoveries of 89.9 ± 6.1 , 93.5 ± 7.6 ; 110 ± 3.1 , and 102 ± 1.0 for 25, 50, 100, and 200ppm ammonia, respectively, when meat samples were vortexed with 0.3M perchloric acid (HClO_4). However, our results showed recoveries $>100\%$ with large coefficients of variation, as shown in Table 5. Results obtained by vortexing spiked meat samples with nano pure water (Table 6) showed average recoveries of 93.2, 80.6, and 87.3 for 50, 100, and 200ppm, respectively, which did not exceed 100%. Perchloric acid is a strong oxidizing agent which might hydrolyze and break down the meat proteins, peptides or amino acids, thereby producing extra ammonia from the meat in addition to the background and spiked ammonia in the sample. Based on Figure 7, the ammonia background of the meat sample extracted using perchloric acid was increased by approximately 50% compared to the ammonia background of meat extracted using nano pure water. Some amino acids, especially asparagine (Asn) and glutamine (Gln), have

been found to release ammonia during acid hydrolysis (Garrett and Grisham, 2010; Creighton, 1993). The amount of ammonia released has been used as a method to estimate the total number of Asn and Gln residues present in a protein (Garrett and Grisham; Creighton, 1993).

Spiking Level (uL)	Ammonia Concentration in Meat (ppm)	Average Recovery of Ammonia (%)	%CV
25	25	234.2 \pm 95.8	40.9
50	50	191.8 \pm 25.4	13.3
100	100	131.3 \pm 30.8	23.5
200	200	151.3 \pm 15.7	10.4

Table 5. Recovery (%) and coefficient of variation (%CV) of ammonia in spiked meat samples (n=4) extracted by vortexing meat with 0.3M perchloric acid (HClO₄) and measured by ammonia selective electrode (ISE). A set of standards (1, 10, and 50ppm) diluted with 0.15M perchloric acid (HClO₄) was used to construct the standard curve. The recovery of ammonia in each sample was calculated by subtracting the ammonia background (control) from the total ammonia concentration in the sample.

Spiking Level (uL)	Ammonia Concentration in Meat (ppm)	Average Recovery of Ammonia (%)	%CV
50 ¹	50	89.6 \pm 34.8	38.9
100 ²	100	84.4 \pm 23.1	21.4
200 ³	200	81.2 \pm 15.9	19.6

Table 6. Recovery (%) and coefficient of variation (%CV) of ammonia in spiked meat sample extracted by vortexing meat with nano pure water and measured by ammonia selective electrode (ISE). A set of standards (1, 10, and 50ppm) diluted in nano pure water was used to construct the standard curve. The recovery of ammonia in each sample was calculated by subtracting the ammonia background (control) from the total ammonia concentration in the sample. ¹n = 21; ²n = 10; ³n = 32.

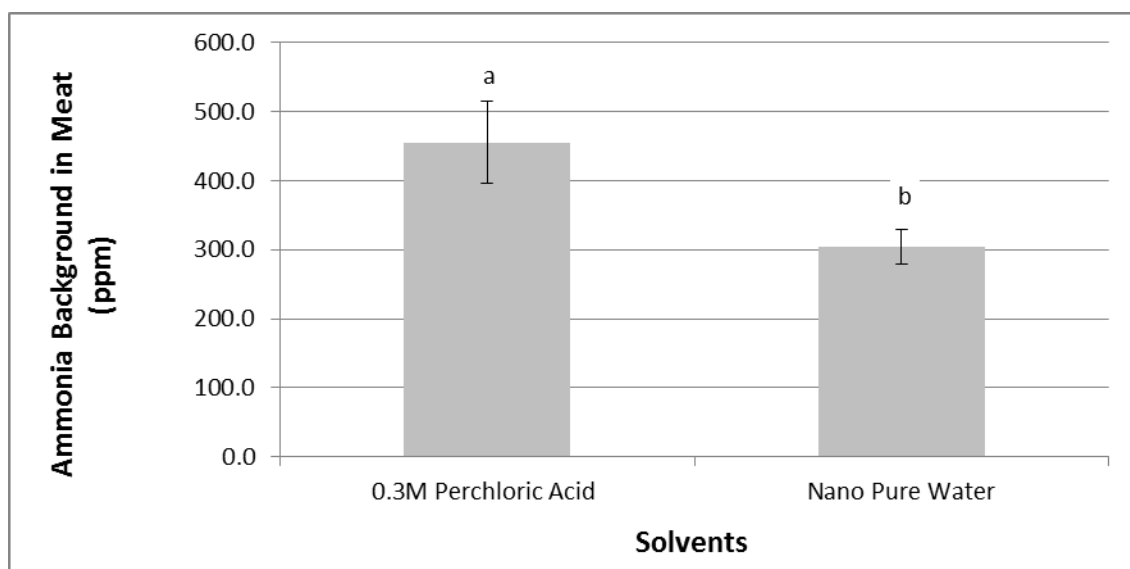


Figure 7. Ammonia background in meat (ppm) extracted by vortexing the meat with different solvents - 0.3M perchloric acid (n=6) or nano pure water (n=62). A set of standards (1, 10, and 50ppm) diluted with the solvents were used to construct the standard curves. The ammonia background was measured by ion selective electrode (ISE). Means with different letters are significantly different at $P<0.05$.

Although vortexing spiked meat samples with nano pure water (Table 6) did not give recoveries exceeding 100%, it had high coefficients of variation ranging from 19.6 to 38.9%. High CVs were observed because ground meat clumped together during vortexing in water, possibly due to the hydrophobic effect, which might undermine ammonia extraction from the meat and lead to variation. Thus, blending spiked meat samples with nano pure water (Table 7) was tested. Blending samples with water gave good %recoveries ranging from 88.7 ± 8.6 to 108.1 ± 10.5 and more consistent results with %coefficients variation ranging from 8.4 to 20.4. The results of blending meat samples with distilled water obtained by Pivarnik et al (1998) also showed similar consistency with %recoveries ranging from 83 to 98.6 and %CV ranging from 4.49 to 17.2. Blending broke the meat matrix and homogenized the meat sample effectively, which allowed the ammonia to be released into the solvent.

Spiking Level (μL)	Ammonia Concentration in Meat (ppm)	Average Recovery of Ammonia (%)	%CV
100	10	108.1 ± 10.5	9.7
200	20	107.5 ± 21.9	20.4
500	50	88.7 ± 8.6	9.7
1000	100	91.4 ± 7.6	8.4
2000	200	94.5 ± 8.9	9.4

Table 7. Recovery (%) and coefficient of variation (%CV) of ammonia in spiked meat (n=2) extracted by blending meat sample with nano pure water and measured by ammonia selective electrode (ISE). A set of standards (1, 10, and 25ppm) diluted with nano pure water was used to construct the standard curve. The recovery of ammonia in each sample was calculated by subtracting the ammonia background (control) from the total ammonia concentration in the sample.

Since the pH of water is prone to change due to exposure to CO₂ from surrounding air, which might affect the results, we tested different pH of solvents for ammonia recovery in the spiked meat sample solutions using ion-exchange chromatography. Extraction using low pH (pH<2) solvents caused hydrolysis and precipitation of meat proteins which led to cloudiness in the final sample solution. An extensive centrifugation process was required to remove the precipitated proteins. Cloudy sample solutions gave problems in IC analysis. In addition, low pH meat extracts, especially using perchloric acid, might shorten the life-span of the ammonia selective electrode. During the period of testing low pH samples, it was necessary to replace the electrode twice. Based on data shown in Figure 8, pH 6 nano pure water had the largest peak area compared to the control (nano pure water without pH adjustment), and was the only treatment significantly different from the control ($P<0.05$). The recovery of ammonia in spiked meat extracted with pH 6 nano pure water was further compared with 0.02M potassium phosphate buffer at pH 5.8, 6.0, 6.2, 6.5, 6.8, 7.0, and 7.2, based on

peak areas determined by the IC method (Figure 9). Statistically, there was no significant difference between pH 6 nano pure water and 0.02M potassium phosphate buffer at pH 6.0 ($P<0.05$). The pH 6 PPB was preferred because of the ease of pH adjustment. The level of ammonia recovered decreased slightly as the pH increased, but only pH 7.2 PPB was significantly different ($P<0.05$). Extraction with a higher concentration of PPB (0.1M) gave cloudy meat extracts, and high potassium ion concentrations in the extracts might pose a problem in ammonia determination by ion chromatography, since the retention time of potassium and ammonia are close to each other. Thus, a lower concentration of pH 6 PPB (0.01M) was preferred, since the main purpose of the buffer was to maintain the pH of the solvent at the preferred pH in order to reduce any variation caused by absorption of CO_2 from the surrounding air. Using pH 6 0.01M PPB as the solvent also helped to solubilize ammonia in the solvent and suppress its escape into the surrounding air, especially during the filtration step. Hijaz et al (2007) stated that filtration reduced the recovery of ammonia in spiked meat samples by 10%.

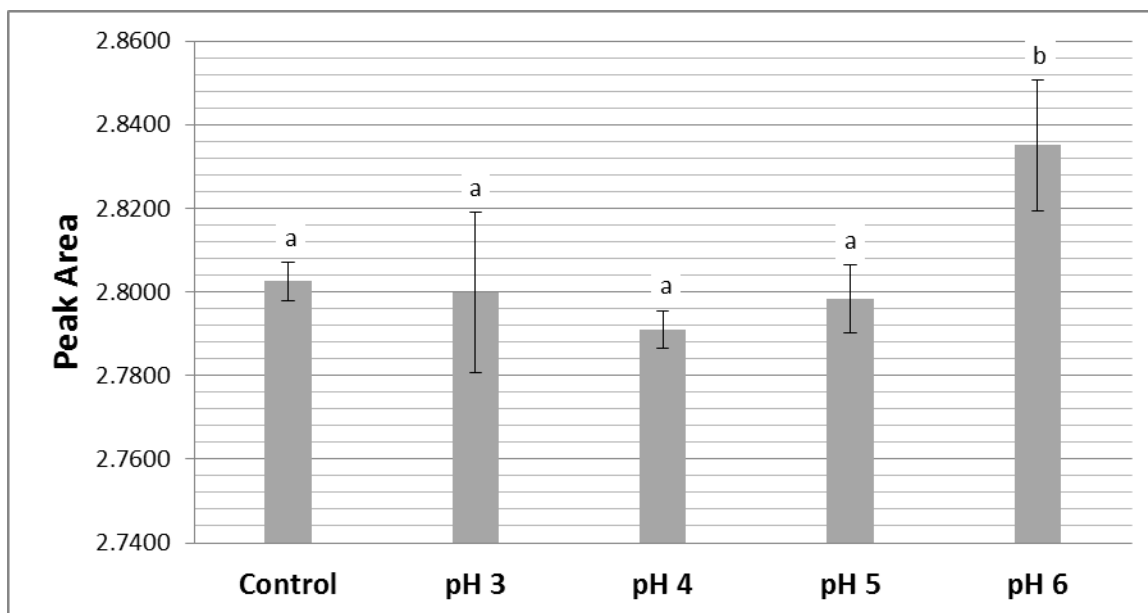


Figure 8. The peak area of 20ppm ammonia in spiked meat (n=3) extracted by blending ground meat samples with nano pure water (control) or nano pure water adjusted to pH 3, 4, 5, or 6 with 0.15M perchloric acid/0.01N NaOH. Extracts were measured by ion-exchange chromatography. Means with different letters are significantly different at $P<0.05$.

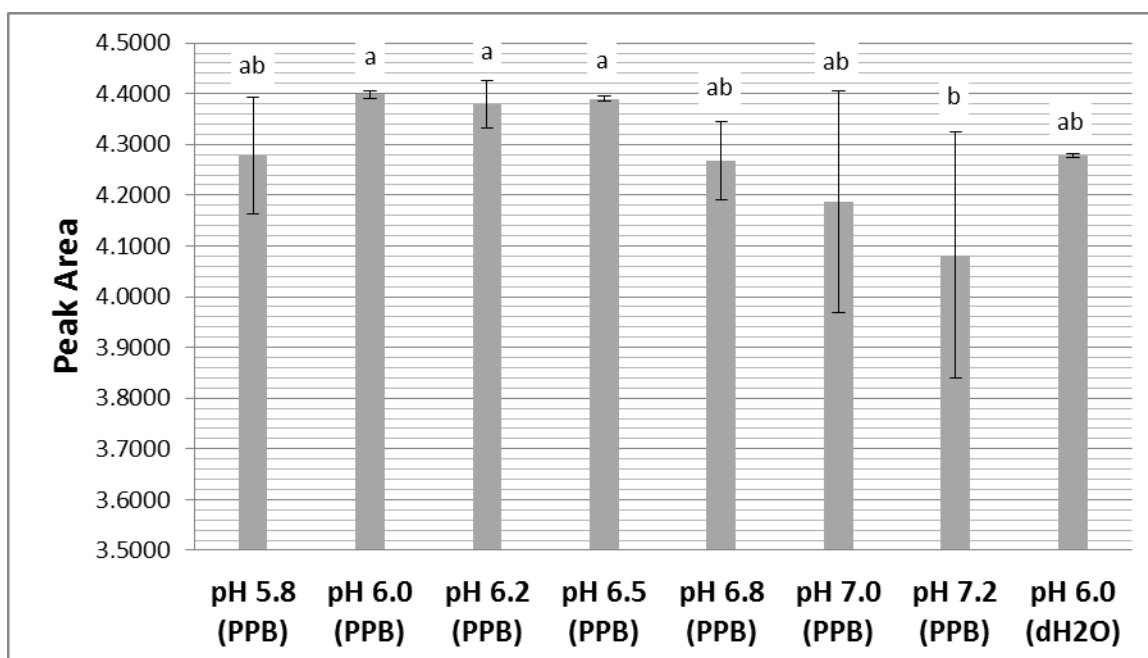


Figure 9. The peak area of 200ppm ammonia in spiked meat (n=2) extracted by blending ground meat samples with 0.02M Potassium Phosphate Buffer (PPB) or nano pure water adjusted to pH 6 with 0.15M perchloric acid/0.01N NaOH. Extracts were measured by ion-exchange chromatography. Means with different letters are significantly different at $P<0.05$.

The ion selective electrode (ISE) method has been found to have a good correlation with the ion-exchange chromatography (IC) method (Proelss and Wright, 1973). The correlation of ammonia levels between ISE and IC was 0.994 (Proelss and Wright, 1973). Our results showed no significant differences between the two methods on ammonia concentrations of 10 to 100ppm ($P<0.05$). The ISE method showed higher standard deviations compared to the IC method (Table 8). However, after using pH 6 0.01M potassium phosphate buffer (PPB) as the solvent in the extraction process, a reduction in the %coefficients of variation were observed as shown in Table 9 compared to Table 7. The % average recovery of ammonia in spiked meat was 91% or above for all concentrations. The % CVs were acceptable except at low concentrations, where the %CVs were higher. This may occur because the levels of ammonia in the meat extracts were close to the detection limit of the electrode. This extraction procedure also allowed the ISE to measure >100 samples without needing to replace the membrane.

Spiking Level (μ L)	Ammonia Concentration in Meat (ppm)	Recovered Ammonia (ppm)	
		Methods	
		IC	ISE
100	10	10.4 ± 0.7^a	10.8 ± 1.1^a
200	20	18.7 ± 0.2^b	21.5 ± 4.4^b
500	50	41.5 ± 4.0^c	44.4 ± 4.3^c
1000	100	81.2 ± 1.9^d	91.4 ± 7.6^d
2000	200	149.7 ± 5.8^e	188.9 ± 17.8^f

Table 8. The average ammonia concentration \pm standard deviation in spiked meat samples (n=2) extracted by blending meat sample with nano pure water measured by ammonia selective electrode. A set of standard (1, 10, and 25ppm) diluted with nano pure water were used to construct the standard curve. The extraction procedure was adapted from Hijaz et al (2007). The level of ammonia was determined by both ion-exchange chromatography (IC) and ion selective electrode (ISE). The recovery of ammonia in each sample was calculated by subtracting the ammonia background (control) from the total ammonia concentration in the sample. Means in rows with different superscript letters are significantly different at $P<0.05$.

Spiking Level (μL)	Ammonia Concentration in Meat (ppm)	Average Recovery of Ammonia(%)	%CV
100	10	91.3 ± 12.9	14.1
200	20	90.9 ± 7.5	8.2
500	50	93.6 ± 5.3	5.6
1000	100	93.9 ± 4.3	4.6
1500	150	94.5 ± 3.9	4.1
2000	200	95.0 ± 3.4	3.6

Table 9. Recovery (%) and coefficient of variation (%CV) of ammonia in spiked meat samples (n=9) extracted by blending meat samples with pH 6 0.01M potassium phosphate buffer (PPB) and measured by ammonia selective electrode. A set of standards (1, 10, and 50ppm) diluted with pH 6 0.01M potassium phosphate buffer (PPB) was used to construct the standard curve. The recovery of ammonia in each spiked sample was calculated by subtracting the ammonia background (control) from the total ammonia concentration in the sample.

Using spiked meat extracts to construct a standard curve was tested in this study, but it offered no advantages to the simpler procedure of preparing standards in solvent. In addition, the recoveries of ammonia in spiked meat samples (Table A-2 and Table A-3 in the appendix) obtained by using spiked meat extracts as standards were low at certain levels and with high %coefficients of variation. Thus, this procedure was not pursued further in this study.

Conclusion

Based on the results, the ISE could be a promising method to be used by industry for detecting and quantifying ammonia contamination in meat rapidly and effectively. Blending after grinding the meat muscle was an effective way to extract ammonia out of the meat matrix, in order to get a consistent high recovery. Acid extraction required extensive steps to remove the protein precipitated, because protein precipitation led to cloudiness in the sample solutions which might cause problems in both ISE and IC

determinations. Furthermore, using perchloric acid as a solvent might extract additional ammonia from the tissue and increase the background ammonia. Thus, using pH 6 0.01M PPB to maintain the pH of the solvent and reduce the rate of ammonia lost to the surrounding air, was found to provide the most consistent results.

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Chapter III

Rate of ammonia uptake by fresh and frozen beef exposed to 200ppm ammonia gas at selected times and temperatures

Abstract

A high water content (~75%) and low pH (5.4 to 5.8) cause meat to be susceptible to ammonia contamination. This study monitored the rate of ammonia uptake by vacuum-packaged fresh, and non-packaged fresh and frozen beef exposed to 200ppm ammonia in nitrogen gas at selected times and temperatures. Fresh meat samples exposed at ambient (20-25°C) and refrigeration (3-5°C) temperatures had rapid rates of ammonia uptake. The level of ammonia contamination in fresh meat had a positive linear relationship the the duration of exposure prior to reaching saturation. The rate of ammonia uptake in fresh meat was 58.4 ± 7.1 ppm per hour for 20-25°C during a 6-hour exposure, and 56.4 ± 5.8 ppm per hour for 3-5°C during a 9-hour exposure. After 9hours, the rate decreased to 24.7 ± 4.7 ppm per hour up to 12hours exposure time at 3-5°C, as saturation began to occur. Unlike fresh meat, frozen meat had a slower ammonia uptake rate. After 12hours exposure at freezing temperature (-13°C), the average ammonia level in frozen meat samples was only 96.0 ± 5.4 ppm, which was approximately 6 times lower than the ammonia level in fresh meat (586.7 ± 14.2 ppm). Moreover, 2.4mil Cryovac type B6620 packaging film provided a good barrier to ammonia gas. No significant difference ($P < 0.05$) was observed in ammonia concentration (ppm) between non-exposed (control) and vacuum-packaged meat samples exposed to 200ppm ammonia at 3-5°C for 12hours. Non-packaged meat samples were found to be contaminated with a total of 688.6 ± 2.1 ppm ammonia.

Introduction

Ammonia has a high affinity for water, and is more soluble at low temperature (CSIRO, 2002) and low pH. Therefore, food products with a high water content, acidic pH (Arnold, 1993), and that are stored at low temperature will be susceptible to ammonia contamination during ammonia refrigerant leakage in food plants. Meat is one of the food products that are susceptible to ammonia contamination. It has a high water content with an average composition of 75% water, 20% protein and 5% combined fat, carbohydrate and minerals (USDA, 2007). Fresh meat also has an acidic pH, ranging from 5.4 to 5.8 under normal conditions. Those two factors (water and pH) make meat susceptible to ammonia contamination.

Ammonia contamination in beef can affect muscle quality including color (Shaw and others, 1992; Al-Sahal, 2003), flavor (Hagyard, 1993; CSIRO, 2002), pH (Anil, 1971; Al-Sahal, 1998; Al-Sahal 2003), odor (CSIRO, 2002), water holding capacity (Anil, 1971; Al-Sahal, 1998; Al-Sahal 2003), and tenderness (Anil, 1971). The degree of ammonia contamination in food products depends on ammonia concentration, temperature, exposure duration, product, and packaging (Arnold, 1993). The concentration of ammonia in an exposed beef sample has been found to have a positive relationship with the duration of exposure and the ammonia level in the surrounding air (Al-sahal, 2003; Karim, 2010). As both exposure time and ammonia level increased, the contamination level of ammonia in meat also increased. According to Arnold (1993), exposure to a low level of ammonia for a longer time has an equal effect on a food product as exposure to a high concentration of ammonia for a short period of time.

Food packaging can affect the rate of ammonia uptake by meat samples. All types of paper packaging such as kraft, waxed paperboard boxes, corrugated fiberboard etc., have high permeability to ammonia (Johnston, 1981) and the ability to hold ammonia in the package. This allows continuous contamination to occur over time, even after the product has been removed from the ammonia leakage environment (CSIRO, 2002). Most types of packaging film may limit or even protect products from ammonia contamination (CSIRO, 2002; Goodfellow, 1978). Basic packaging films, for example, polyethylene, have enough barrier properties to protect food products from damage caused by ammonia contamination (Arnold, 1993). Cryovac E-2300, low density polyethylene (LDPE), and vacuum-polyamide/polyethylene (V-PA/PE) films have been shown to have the ability to protect water from ammonia contamination (at levels 50, 100, 250, and 500ppm) for 2 days exposure at freezing temperature (Karim, 2010).

Although some studies have tested the rate of ammonia contamination in meat products exposed to different ammonia levels, temperatures, and exposure times, there were still some missing information which was not provided in those studies. In addition, packaging films tested in the past were filled with water instead of actual meat. Since water and meat samples have different physical and chemical characteristics, the level of contamination may differ. Therefore, this study was conducted to provide further information that has not been evaluated in the past. One of the objectives of this study was to monitor the rate of ammonia uptake in beef samples exposed to 200ppm NH_3 in N_2 gas at three different temperatures - ambient (20-25°C), refrigeration (3-5°C), and freezing (-13°C), for 1 to 12 hours. A selected packaging film, commonly used to

package fresh beef, was also tested for its permeability to ammonia gas for 12 hours exposure at refrigeration temperature.

Material and Methods

Meat Sample

The cut of beef used in the study was eye of round roast purchased from a local grocery store (HyVee, Lincoln, NE). The meat was stored in a freezer after purchase and before being analyzed, in order to slow down the spoilage process. The eye of round roast (Figure 10) was trimmed and fabricated into a 2.5x2.5x1 inch size (6.35 X 6.35 X 2.5 cm) (Length x Width x Height) a day prior to analysis. Another meat piece, which was trimmed from the same eye of round roast, was stored under the same conditions, and was used to provide the ammonia background (control).



Figure 10. Fabricated eye round roast with dimension of 2.5x2.5x1 inch (Length x Width x Height)

Exposure System

The exposure system consisted of gas cylinder containing 200ppm ammonia (NH₃) in nitrogen (N₂) gas, a gas pressure regulator, a gas flow rate regulator, an exposure chamber, a refrigerator/freezer, and an ammonia monitor (Figure 11). The parts

of the exposure system were connected by Tygon® B-44-3 tubing. The certified 200ppm ammonia (NH₃) in nitrogen (N₂) gas, gas pressure regulator, and Matheson Tri-Gas gas flow rate regulator were purchased from Linweld, Inc, a division of Matheson Tri-Gas. The refrigerator/freezer unit had a thermostat which allowed temperature settings ranging from -13 to 5°C.

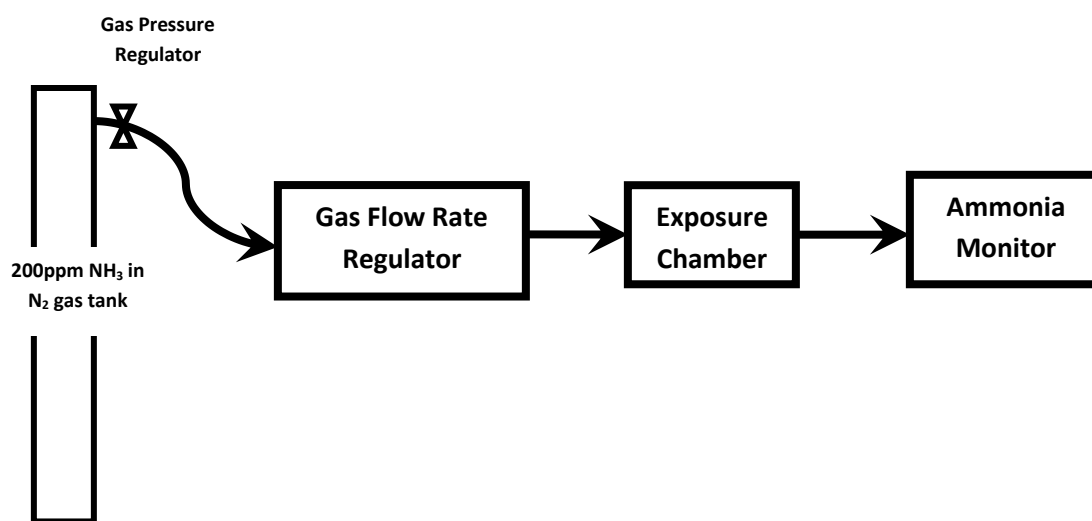


Figure 11. The ammonia gas exposure system

A mini desiccator cabinet (Secador®) from Bel-Art Products (Pequannock, NJ) was used as the exposure chamber (Figure 12). The desiccator cabinet had an overall volume of 18 liters and dimensions of 13.3 x 10 X 8.5inch (33.8 X 25.4 X 21.6cm) (L x W x H) with a 9.3 x 5 inch (23.6 X 12.7cm) (L x H) door opening secured by a latch against a polymer seal. The small size of the desiccator minimized the amount of gas needed to purge the interior, and it fit well in the refrigerator/freezer unit. The desiccator

had two gas ports which allowed exchange of gases. A hook was attached in the middle of the exposure chamber to allow the meat sample to hang in the cabinet during exposure.



Figure 12. Mini desiccator cabinet (Secador®) with an overall dimension of 13.3 x 10 X 8.5 (L x W x H) inch and door opening of 9.3 x 5 (L x H) inch

An EAGLE™ ammonia monitor was purchased from RKI instruments (Union City, CA). This detector was used to monitor the level of ammonia gas inside the chamber during exposure. It measured the ammonia level in parts per million (ppm), and was calibrated monthly with a 25ppm ammonia (NH₃) in nitrogen (N₂) standard gas prepared by Linweld.

Reagents

Potassium phosphate buffer (PPB). 0.01M, pH 6, potassium phosphate buffer (PPB) was used as the solvent to extract ammonia from exposed meat samples. It also was used to prepare the ammonia standard solutions. The buffer was prepared by combining and diluting 1M monobasic and 1M dibasic potassium phosphate, and adjusting the pH of the buffer to the desired value with 0.01M phosphoric acid. The pH was measured with an Orion 9145BN pH electrode connected to an Orion 2-Star

benchtop pH meter. The pH meter was calibrated using pH 4 and pH 7 Orion standardization buffers.

Ammonia Standards. A series of ammonia standard solutions (1, 10, 50, and 100ppm) was prepared by diluting a 1000ppm commercial ammonia standard solution (Ricca Chemical Company) with 0.01M PPB at pH 6. 100mL of each standard solution was freshly prepared daily and used to construct a standard curve. A standard curve was constructed by plotting the milli-volt (mV) readings against the logarithm of the ammonia standard concentrations. The levels of ammonia in exposed meat samples were determined by using a regression equation obtained from the standards.

pH-adjusting ISA. A commercial pH-adjusting ionic strength adjuster (ISA) solution was purchased from Thermoscientific (Cat. No. 951211). The same amount was added into each standard and sample solution to raise the pH of the sample and standard solutions to an alkaline value. 1.6mL of pH-adjusting ISA was added into 50mL of sample or standard solution to raise the pH of the solution to above 11. At alkaline pH, ammonium ion in the solution is converted into ammonia gas and can be measured by the ammonia selective electrode.

Packaging Film

Cryovac type B6620 film bags were selected to be tested for ammonia gas permeability. These packaging bags were provided by Sealed Air Corporation, Duncan, SC. The specification of Cryovac type B6620 film is provided in the appendix (Table A-4).

Ammonia Selective Electrode (ASE) and pH meter

The level of ammonia in a sample was determined using an ammonia selective electrode, model Orion 9512HPBNWP from Thermo Scientific. The ammonia selective electrode was set up, maintained and stored according to the user guide manual provided by the manufacturer. It was connected to an Orion model 420A pH meter, and the meter was set to read in milli-volts (mV).

Experimental Design

Fresh and Frozen Meat Samples without Packaging Film

- Exposure Procedure

A fabricated meat sample (2.5x2.5x1 inch) was hung in the middle of the exposure chamber and exposed to 200ppm of ammonia gas in N₂ at one of three different temperatures - ambient (20-25°C), refrigeration (3-5°C), or freezing (-13°C), for a specific length of time. The fabricated eye of round roast was thawed overnight in a refrigerator (3-5°C) for exposure at ambient and refrigeration temperatures. In the case of frozen temperature analysis, the fabricated beef was stored in the freezer (-13°C) overnight before exposure, and it was maintained in the frozen state while being exposed to ammonia.

The chamber containing a meat sample was flushed with 200ppm NH₃ gas at 4psi and maximum flow rate for 4 minutes. The purpose of flushing was to flush out the air inside the chamber and allow the NH₃ concentration inside the chamber to equilibrate. After flushing, the gas flow rate was adjusted to 25mL/sec and the exposure timing was started. At ambient temperature (20-25°C), meat samples were exposed to ammonia gas from 1 to 6 hours. Longer exposure times at 20-25°C were not used due to microbial and

enzymatic action that could contribute to increasing ammonia levels. At both refrigeration (3-5°C) and freezing (-13°C) temperatures, the exposure times were 1, 2, 4, 6, 9, and 12 hours. Each treatment was replicated three times.

- Extraction Procedure

After exposure to NH_3 gas, the ammonia was extracted from each contaminated meat sample. Meat samples exposed at both ambient and refrigeration temperatures were extracted immediately after exposure. The frozen meat samples exposed at freezer temperature were thawed overnight and then extracted on the next day. The extraction procedure was adapted from Hijaz et al (2007) with some alteration. The contaminated meat was ground three times using a bench-top Oster heavy duty meat grinder. $10 \pm 0.05\text{g}$ of ground meat was blended with 90mL pH 6 0.01M potassium phosphate buffer (PPB) at high speed for 30 seconds. The blended meat solution was split into two-50mL centrifuge tubes and was centrifuged (IEC Centra CL2 centrifuge) at $1000 \times g$ for 10 minutes. Most of the ammonia present in the meat will be in the form of ammonium ion. Blending helps release the ammonium ion from the meat matrix into the buffer solvent. Centrifugation helps to spin down all the meat particles and fat. The two-50mL supernatants were combined and filtered through Whatman No. 4 filter paper to further remove the particles and fat that did not spin down by centrifugation. During the filtration process, a watch glass was placed over the funnel to prevent ammonium ion from escaping as ammonia gas into the air. The filtrate was adjusted to 100mL with nano pure water. The unexposed trimmed meat piece (control) was also extracted in the same way as the exposed meat sample. Since the meat originally contained a certain amount of

ammonia, this served as an ammonia background which was subtracted from the total ammonia level in the exposed meat sample to obtain the level of ammonia contamination.

- Ammonia Determination by Ion Selective Electrode (ISE)

A 50mL aliquot of the 100mL of standard solution or filtrate was transferred into a 100mL beaker. Measurement of ammonia was conducted according to the ISE user guide by adjusting the ionic strength and pH of each extract or standard using pH-adjusting ISA solution. As soon as the electrode was placed into the 50mL standard solution or filtrate, the pH of the solution was brought to above 11 by addition of 1.6mL of pH-adjusting ISA and was mixed evenly with a magnetic stir bar at constant moderate speed. The mV was recorded once it was stable. Measurements were made in order starting with standards (low to high), control and samples. The background ammonia level (control) was subtracted from the total amount of ammonia in the sample solution to obtain the actual ammonia contamination in the exposed meat.

Vacuum Packaged Fresh Meat in Cryovac B6620 film

The fabricated frozen meat samples (2.5x2.5x1 inch) and the control frozen meat pieces (Figure 13) were vacuum-packed separately in Cryovac type B6620 film bags using a vacuum packager (Multivac model C500 manufactured in Germany). All samples were packaged at the Animal Science Department Meat laboratory (University of Nebraska-Lincoln, Lincoln, NE).

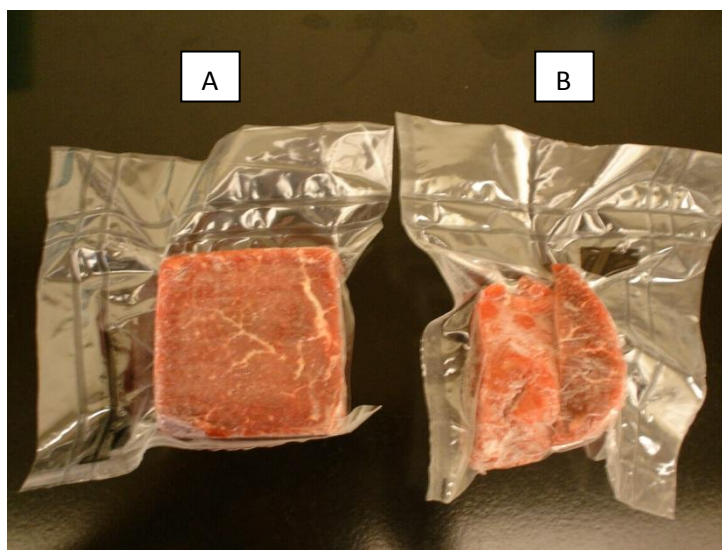


Figure 13. Meat vacuum-packed in Cryovac type B6620 film. A: Meat sample (2.5x2.5x1 inch) for ammonia exposure; B: Control - meat pieces cut from same piece of meat as A.

A vacuum-packaged sample was exposed to 200ppm ammonia in N_2 gas for 12hours at refrigeration temperature (3-5°C). It was thawed overnight in a refrigerator before starting the exposure process. The procedures of exposure, extraction, and determination of ammonia levels for vacuum-packed samples were similar to non-vacuum-packaged meat samples at refrigeration temperature. A non-packaged fresh meat sample was also exposed to ammonia gas along with the vacuum-packed sample for comparison. After the exposure process, both non-packaged and packaged samples were extracted and analyzed by ISE on the same day. The whole treatment was replicated three times. The amount of ammonia in both vacuum-packaged meat and non-packaged meat samples were reported without subtracting the background ammonia level (control).

Statistical Analysis

The data were analyzed using the SAS program (Version 9.2, SAS Inst. Inc., Cary, N.C., U.S.A., 2002). PROC MIXED was used to analyze significant differences in ammonia

uptake among vacuum-packaged fresh, non-packageed fresh and frozen meats exposed to 200ppm ammonia in nitrogen gas at selected times and temperatures.

Results and Discussion

Rate of ammonia uptake in fresh and frozen meat without packaging

Exposing fresh meat to 200ppm NH_3 in N_2 gas at ambient (20-25°C) and refrigeration (3-5°C) temperatures showed a rapid increased in ammonia concentration in meat (Figure 14). The ammonia level in fresh meat at 20-25°C was higher than fresh meat at 3-5°C during first 1 to 3 hours of exposure. After 4 hours exposure, the ammonia levels in fresh meat at both temperatures showed no significant differences at $P<0.05$. The rate of NH_3 uptake in fresh meat exposed at both ambient ($R^2=0.9983$) and refrigeration ($R^2=0.9993$) temperatures had positive linear relationships (Figure 15) between ammonia concentration and time of exposure before reaching saturation. Al-Sahal (2003) and Karim et al (2010) stated that as the time of exposure increased, the amount of ammonia absorbed into the meat also increased.

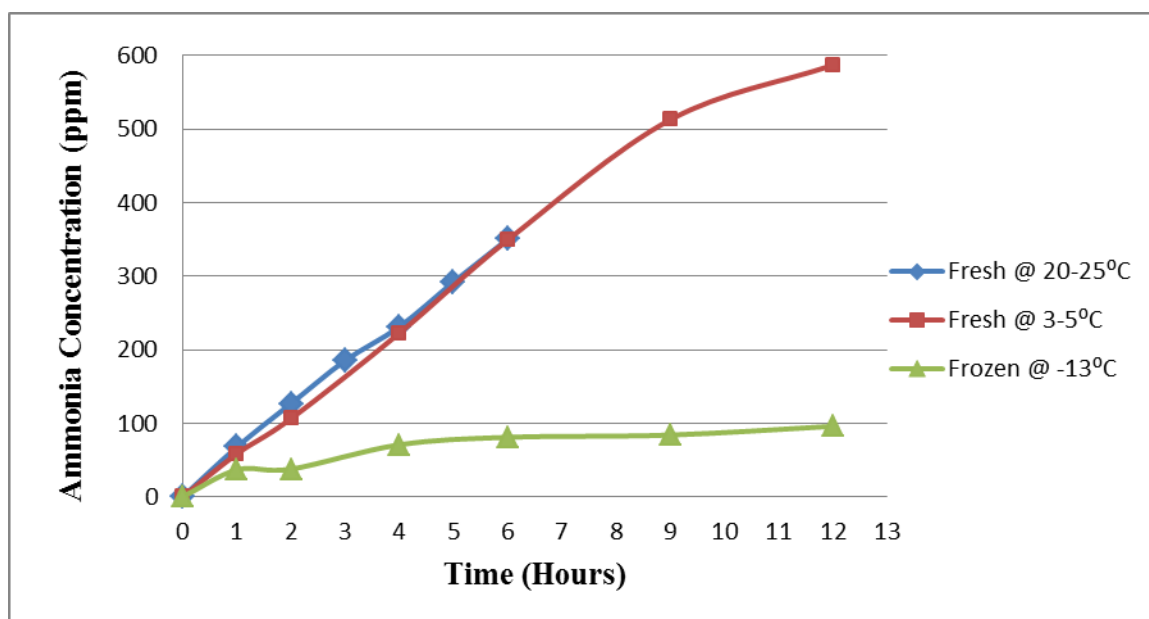


Figure 14. NH_3 concentration (ppm) in fresh meat at ambient (20-25°C) and refrigeration (3-5°C) temperatures, and frozen meat at freezer temperature (-13°C), at varying exposure times

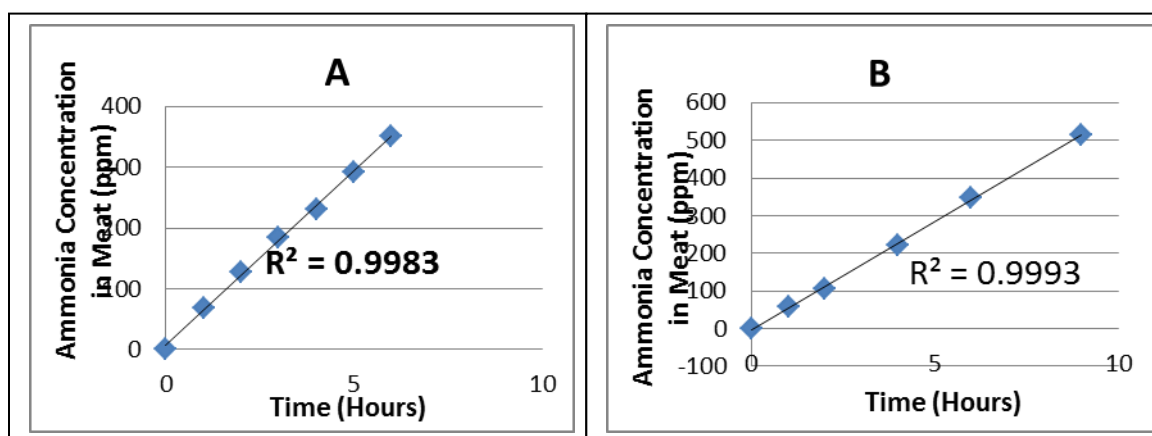


Figure 15. Correlation between ammonia level in meat and exposure time. A: fresh meat exposed to 200ppm NH_3 at ambient (20-25°C) temperature; B: fresh meat exposed to 200ppm NH_3 at refrigeration (3-5°C) temperature

In this study, the level of ammonia in fresh meat increased at a rate of 58.4 ± 7.1 and 56.4 ± 5.8 ppm per hour for ambient and refrigeration temperatures, respectively, before reaching saturation. A sign of saturation was observed after exposing fresh meat for more than 9 hours at refrigeration temperature (Figure 14); as the rate of ammonia uptake by fresh meat between 9 and 12 hours of exposure increased more slowly at 24.7 ± 4.7 ppm per hour. Al-Sahal (2003) exposed meat samples to a higher level of ammonia (500 ppm) for short periods of time (0, 5, 10, and 20 minutes) at ambient temperature. His results showed an increase of 43.6 ppm ammonia in meat samples after 5 minutes exposure. Higher levels of ammonia contamination were observed in the Al-Sahal study because thinner meat samples (0.9 cm) and higher levels of ammonia gas (500 ppm) were used in his study. Thinner meat had a larger surface area exposed to the gas; thus, the contamination rate increased faster in a short period of time. In addition, there was a correlation between ammonia concentration in a meat sample and the ammonia concentration level to which the meat was exposed (Karim, 2009; Karim et al., 2010; Al-Sahal, 2003). As the ammonia gas concentration increased in the surrounding air, the amount of ammonia absorbed in the meat was also increased (Karim, 2009; Karim et al., 2010; Al-Sahal, 2003).

Time (Hours)	Ammonia Concentration in Meat (ppm) ¹		
	Fresh at 20-25°C	Fresh 3-5°C	Frozen at -13°C
1	67.8 ± 2.0 ^a	58.6 ± 4.4 ^g	36.6 ± 0.8 ^m
2	126.7 ± 4.0 ^b	106.3 ± 3.6 ^h	37.7 ± 1.6 ^m
3	185.2 ± 3.0 ^c	ND ²	ND ²
4	231.2 ± 6.0 ^{d**}	222.5 ± 9.9 ^{i**}	70.8 ± 7.6 ⁿ
5	292.1 ± 10.1 ^e	ND ²	ND ²
6	350.2 ± 14.3 ^{f**}	349.1 ± 6.1 ^{j**}	80.9 ± 5.7 ^o
9	ND ²	512.6 ± 11.8 ^k	84.0 ± 4.0 ^o
12	ND ²	586.7 ± 14.2 ^l	96.0 ± 5.4 ^p

Table 10. Ammonia concentration in fresh and frozen meats exposed to 200ppm NH₃ for 1, 2, 3, 4, 5, 6, 9, and 12 hours at ambient (20-25°C), refrigeration (3-5°C) and freezer (-13°C) temperatures. ¹ Value are means ± standard deviation of 3 replicates. ² The values were not determined. ^{a-p}Means with different superscript letters within a column are significantly different at P<0.05. ^{**}Means in a row are not significantly different at P<0.05.

The process of ammonia uptake by meat samples was not solely controlled by simple diffusion. In Table 10, it shows that the levels of ammonia in the fresh meat samples exposed to 200ppm NH₃ for 4 hours or longer were >200ppm. This meant that the meat had the ability to absorb ammonia from the surrounding air and to keep accumulating ammonia in its matrix until it reached saturation at levels above the atmospheric concentration. Thus, longer exposure times could lead to much higher concentrations of ammonia contamination in exposed meat which exceed the concentration of NH₃ to which it was exposed.

Exposing frozen meat to 200ppm NH₃ in N₂ gas at freezer temperature showed a slower rate of ammonia uptake by the meat. Table 10 shows that at 12 hours exposure, the concentration of ammonia in frozen meat only reached 96.0±5.4ppm, which was approximately 6 times lower than the ammonia level in fresh meat at 12hours exposure. Karim et al (2010) also observed a slower ammonia contamination rate when he exposed frozen meat (10x5x2.3cm) to ammonia levels of 50, 100, 250 and 500ppm for 0, 6, 12, 24

and 48 hours at $-17\pm3^{\circ}\text{C}$. According to CSIRO (2002), even though ammonia gas is three times more soluble in ice at -30°C than in water at 0°C , the diffusion rate of ammonia into the frozen tissue is slower than into non-frozen tissue. This may be due to the rigid structure of the frozen tissue which slows down the penetration and diffusion of ammonia into the meat. Thus, the state of the meat (frozen vs. fresh) may affect the rate of ammonia uptake in meat. Hagyard (1993) also stated that frozen meat had slower ammonia uptake compared to fresh meat. On the other hand, temperature may not affect the rate of ammonia uptake by fresh meat. Based on statistical analysis (Table 10), there were significant differences in the ammonia levels between fresh meat exposed at ambient and refrigeration temperatures for 1 to 2 hours, but there were no significant differences between in the ammonia levels them after 4 hours.

Ammonia uptake in vacuum-packaged fresh meat

Our results showed that the Cryovac type B6620 film bags have good barrier properties to ammonia gas at refrigeration temperature ($3-5^{\circ}\text{C}$). Comparing ammonia concentration in both unexposed (control) and vacuum-packed meat samples (Table 11 and Figure 16), there was no significant change observed between the two meat samples ($P<0.05$). The non-packaged meat samples showed high levels of ammonia uptake ranging from 687.2 ± 0.9 to $690.1\pm6.7\text{ppm}$. This showed that the Cryovac B6620 packaging film has the ability to protect the meat sample from ammonia gas contamination.

Meat Samples	Ammonia Concentration in Meat (ppm)		
	1	2	3
Control ¹	108.7 ± 0.7 ^a	106.5 ± 0.9 ^a	104.1 ± 1.4 ^a
V-packaged ¹	110.1 ± 0.1 ^{a**}	105.3 ± 1.2 ^{a**}	105.9 ± 2.8 ^{a**}
Non-packaged ²	ND ³	690.1 ± 6.7 ^{b**}	687.2 ± 0.9 ^{b**}

Table 11. Ammonia concentration in control, vacuum-packaged, and non-packaged meats exposed to 200ppm NH₃ for 12 hours at refrigeration (3-5°C) temperature. ¹Values are means ± standard deviations of 3 replicates. ²Values are means ± standard deviations of 2 replicates. ³The values were not determined. Means in rows with different superscript letters are significantly different at $P<0.05$. **Ammonia concentration (ppm) reported without subtracting the ammonia background (control)

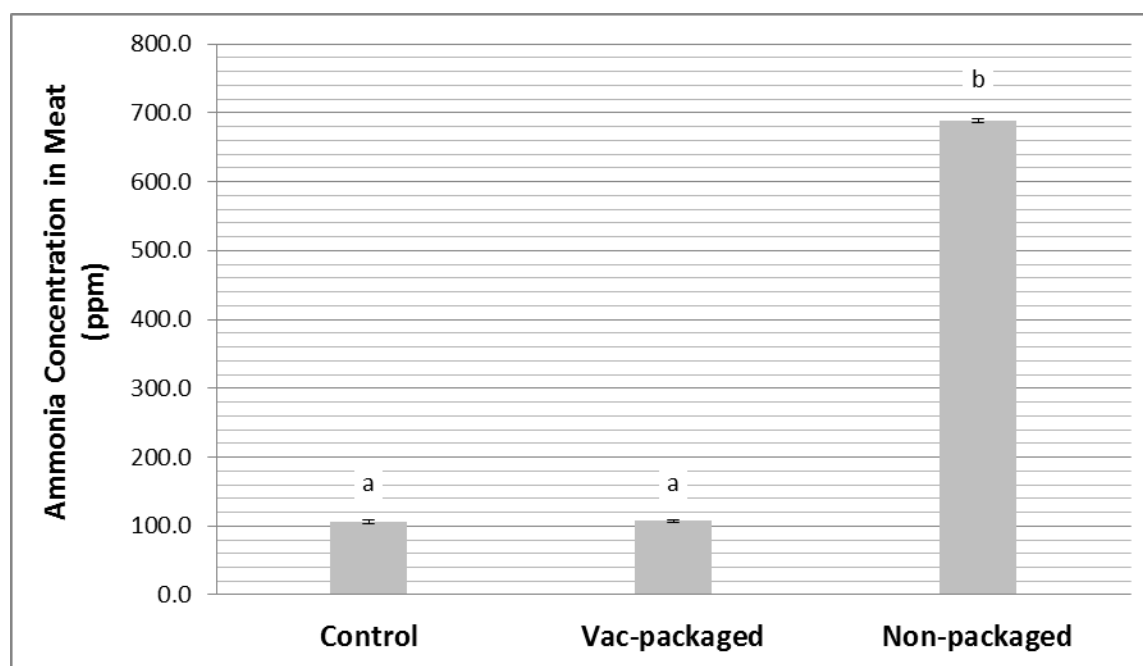


Figure 16. Ammonia concentration in control, vacuum-packaged, and non-packaged meat samples. Means with different superscript letters are significantly different at $P<0.05$

Karim et al (2011) tested three different types of packaging film bags (Cryovac E-2300, vacuum polyamide/polyethylene (V-PA/PE), and low density polyethylene (LDPE)) filled with water for their permeability to ammonia gas at both ambient and freezer temperatures. He concluded that there were no significant increases in ammonia

level in any of the three water pouches during ammonia exposure at freezing temperature; however, low levels of ammonia contamination were observed in all three types of water pouches when exposed to ammonia gas at room temperature. The maximum levels of ammonia observed in the water packed in Cryovac E-2300, LDPE and vacuum PA/PE after 2-day exposures were 7.77 ± 0.67 , 5.94 ± 0.16 , and 0.89 ± 0.20 ppm, respectively.

Conclusion

Meat has the ability to absorb, trap and concentrate ammonia in its system. The ammonia concentration in exposed meat was found to reach a level which was higher than the level of ammonia gas in the surrounding atmosphere. By the time it reaches a saturation point, the ammonia contamination level in meat may be three times higher than the level of gas it is exposed to. The state of the meat samples (frozen vs. fresh) affected the rate of ammonia uptake greatly. Frozen meat absorbed ammonia at a much slower rate compared to fresh meat. Cryovac B6620 packaging film also affected the ammonia uptake in meat samples by acting as a barrier which prevented penetration of ammonia gas into sample. Packaging films do have the ability to protect meat from ammonia contamination.

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Chapter IV

Investigation of selected techniques for lowering ammonia levels in contaminated meat, including air flushing, vacuum treatment, and rinsing with dilute organic acid solutions

Abstract

Minimal study has been done on methods for reducing ammonia concentration in contaminated meat. This study tested three methods to reduce ammonia contamination. Those methods were air flushing for 1 or 2 hours, vacuum treatment, and 2% acetic acid rinsing. Two similar size fabricated samples from eye of top round roast (2.5x1.25x1.0 inch) were exposed to 200ppm NH_3 in N_2 gas for 4hours at 3-5°C. One of the contaminated meat samples was analyzed for total ammonia concentration, and the other piece was subjected to an ammonia removal process. All samples were measured by ion selective electrode (ISE) assay. All removal methods tested were not effective to reduce ammonia concentration in contaminated meat. The percent reduction obtained was 0.6 ± 3.3 , 3.0 ± 1.7 , 6.6 ± 3.8 , and 8.3 ± 4.4 for 1 and 2 hours air flushing, vacuum treatment, and 2% acetic acid rinsing, respectively. Vacuum treatment and acid rinsing might be effective treatments to reduce ammonia contamination on the meat surface only. Rinsing with 2% acetic acid lowered the pH of the exposed meat from 5.83 ± 0.05 to 5.57 ± 0.05 .

Introduction

Ammonia contamination in meat can be detrimental to both meat quality and human health. Ammonia has been found to affect color (Shaw et al, 1992; Al-Sahal, 2003), flavor (Hagyard, 1993; CSIRO, 2002), pH (Anil, 1971; Al-Sahal, 1998; Al-Sahal 2003), odor (CSIRO, 2002), water holding capacity (Anil, 1971; Al-Sahal, 1998; Al-Sahal 2003), and tenderness (Anil, 1971) of the contaminated meat. Exposure to ammonia gas could increase the concentration of ammonia in meat to a degree that could cause illness among people who consumed the contaminated meat. Contamination levels as low as 500ppm in food products have led to stomachache, headache, nausea, sore mouth and throat, and vomiting among people who consumed the contaminated food products. Cooked chicken tenders contaminated with ammonia ranging from 880ppm to 1,076ppm caused 157 students and teachers to become ill after consuming them (Dworkin et al, 2004). In addition, milk contaminated with ammonia ranging from 530ppm to 1,524ppm caused 20 elementary schoolchildren to become ill within an hour (CDC, 1986).

Cooking effects on ammonia contaminated meat have been evaluated by Nigmatullina (1987) for their effectiveness in reducing ammonia concentration. Three different types of cooking methods, such as boiling, frying, and stewing, have been tested, and Nigmatullina (1987) concluded that both boiling and frying methods were able to reduce ammonia levels in contaminated meat by two fold. However, according to Dworkin et al (2004), heating food contaminated with high amounts of ammonia did not reduce the ammonia concentration to a safe level. Johnston (1981) suggested that melting the water/ice glaze and a washing action might remove ammonia in contaminated frozen

meat since ammonia might only absorb and accumulate in the water glaze on the meat. Furthermore, aeration of ammonia contaminated meat for 30 to 60 minutes has been shown to reduce the pH of contaminated meat (Al-Sahal, 2003). Reduction of pH could also mean reduction of ammonia content in contaminated meat (Al-Sahal, 2003).

Based on the literature, not many studies have been done to try to remove ammonia from contaminated meat; thus, the objective of this study was to investigate selected removal methods (1 and 2hour air flushing, 2hour vacuum treatment, and rinsing with 2% acetic acid) for lowering ammonia levels in contaminated meat samples. Acetic acid was selected as a rinsing solution because it is one of the organic acids which has long been studied and reviewed for its antimicrobial activity in inactivation of pathogenic and non-pathogenic bacteria on carcasses (Dorsa et al, 1997; Bell et al, 1997). According to Bell et al (1997) and Dorsa et al (1997), acetic acid has been approved by the U.S. Department of Agriculture Food Safety and Inspection Service (FSIS) as a sanitizer for beef carcasses prior to chilling.

Materials and Methods

Meat Samples

The cut of beef used in the study was eye of round roast purchased from a local grocery store (HyVee, Lincoln, NE). The meat was stored in a freezer (-18°C) after purchase and before being analyzed in order to slow down the spoilage process. The day before analysis, the eye of round roast was trimmed and fabricated into a 2.5x2.5x1 inch sample (6.35 X 6.35 X 2.5 cm) (Length x Width x Height), and the fabricated meat piece was then divided in half (2.5x1.25x1.0inch) (Figure 17). Half of the meat was used for

determining total ammonia in the contaminated meat after exposure and the other half was subjected to a removal treatment. The meat trimmed from the same piece was stored under the same conditions and used as the ammonia background (control).

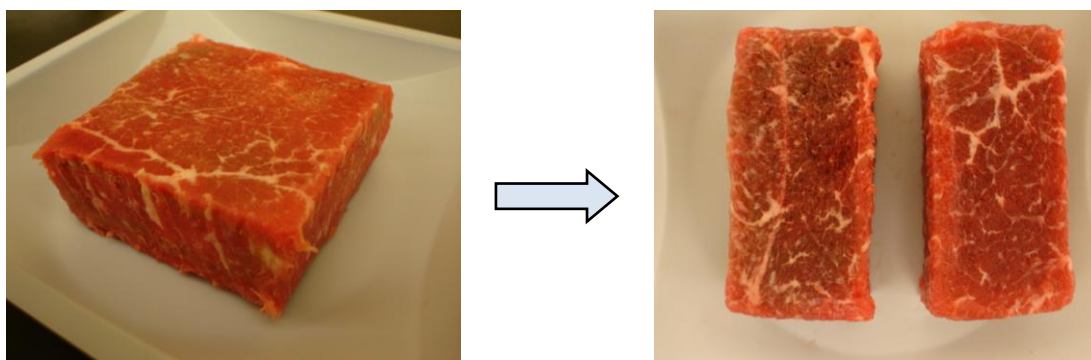


Figure 17. Fabricated eye of round roast with dimension of 2.5x2.5x1 inch (Length x Width x Height) cut into half. Each half has a dimension of 2.5x1.25x1.0 inch

Exposure Procedure

The meat samples were thawed overnight in the refrigerator before exposure. Two meat samples (2.5x1.25x1.0 inch) were suspended in the middle of the exposure chamber (previously described in Chapter 3) and exposed to 200ppm of ammonia in N_2 gas at refrigeration (3-5°C) temperature for 4hours.

The chamber containing the meat samples was flushed with 200ppm NH_3 gas at 4psi and maximum flow rate for 4 minutes. The purpose of flushing was to flush out the air inside the chamber and allow the NH_3 gas inside the chamber to equilibrate. The gas flow rate was then adjusted to 25mL/sec and the timing for 4hours exposure started. After exposure, one of the contaminated meat samples was removed from the exposure chamber for total ammonia concentration determination. The other contaminated meat piece was further processed by air flushing, vacuum treatment, or acid rinsing. This

whole process was repeated to provide three replications of each ammonia removal treatment.

Ammonia removal by air flushing

The contaminated meat piece was suspended inside the chamber for ammonia removal treatment by 1 or 2 hours of clean air flushing. The air flushing treatment was conducted at refrigeration temperature (3-5°C) by placing the chamber into a refrigerator and flushing the inside of the chamber with clean air for 4 minutes at a flowrate of 100mL/sec before starting the 1 or 2 hour air flushing treatment with the same flow rate. The flowrate used resulted in 20 complete exchanges of air inside the chamber in a one hour period. The air was passed through a tube filled with glass wool and bubbled through distilled water before it reached the chamber. This removed particulate matter from the compressed air, and saturated the air with water vapor to minimize surface dehydration of the meat. After 1 or 2 hours of air flushing was completed, the meat sample was extracted and analyzed by the ISE method for ammonia concentration. Three replications were repeated for each treatment time.

Ammonia removal by vacuum treatment

A vacuum desiccator equipped with a vacuum gauge was used in this treatment. The contaminated meat sample was suspended inside the vacuum desiccator for 2 hours of vacuum treatment. The desiccator was vacuumed until the gauge reached 75mmHg inside the desiccator, and it was then placed in a refrigerator. During the 2 hours of vacuum treatment, at an interval of 15 minutes, the desiccator was removed from the refrigerator, a vacuum was pulled for 30 seconds, and then it was placed back into the refrigerator.

After the vacuum treatment, the meat sample was extracted and measured by the ISE method. A total of three replications were repeated for this treatment.

Ammonia removal by acetic acid rinsing

After exposure to 200ppm ammonia in N₂ gas, the meat was rinsed with 2% acetic acid to remove ammonia. The 2% acetic acid solution was prepared by diluting 99.8% acetic acid (from Acros Organics) with de-ionized water. The contaminated meat sample was rinsed twice with 2% acetic acid from top to bottom until the entire surface was covered to dripping. The rinse acid was applied to the contaminated meat sample using a polyethylene wash bottle. The acid rinsed sample was extracted and measured with the ISE method for ammonia. This acid rinse treatment was repeated three times.

pH Determination. The pH of a meat sample was also measured before and after rinsing. The procedure for pH determination in meat samples was adapted from Young et al (2001). 5g of ground meat was blended with 50mL of deionized water using a Waring blender at high speed for 30 seconds. The meat suspension was transferred to a 50mL beaker and stirred constantly at moderate speed with a magnetic stir bar while reading the pH. The pH was measured as soon as possible to reduce any errors due to pH change caused by CO₂ exposure. An Orion 9145BN pH electrode connected to an Orion 2-Star benchtop pH meter was used to measure the pH in the meat sample. The pH meter was calibrated using pH 4 and pH 7 Orion standardization buffers.

Extraction procedure

All meat samples (exposed meat, treated meat and unexposed trimmed meat (control)) were extracted using the procedure adapted from Hijaz (2007) with some alteration. The meat sample was ground three times using a bench-top Oster heavy duty

meat grinder. 10 ± 0.05 g of ground meat were blended with 90mL of pH 6 0.01M PPB at high speed for 30 seconds using a Waring blender. 0.01M (pH 6) potassium phosphate buffer (PPB) was used as the solvent to extract ammonia from exposed meat samples. It also was used as a solvent to prepare the ammonia standard solutions. The buffer was prepared by combining and diluting 1M monobasic and 1M dibasic potassium phosphate, and the pH of the buffer was adjusted to the desired pH using 0.01M phosphoric acid. The pH of the buffer was measured by an Orion 9145BN pH electrode connected to an Orion 2-Star benchtop pH meter. The pH meter was calibrated using pH 4 and pH 7 Orion standardization buffers.

The blended meat solution was split into two-50mL centrifuge tubes and was centrifuged (IEC Centra CL2 centrifuge) at 1000x g for 10 minutes. Most of the ammonia present in the meat will be in the form of ammonium ion. Blending will help release the ammonium ion from the meat matrix into the buffer solvent, and the centrifugation step helps to spin down all the meat particles and fat to ease the filtration. The two-50mL supernatants were combined and filtered through Whatman No. 4 filter paper to further remove the particles and fat that did not spin down by centrifugation. During the filtration, a watch glass was placed over the funnel to reduce the rate of ammonium ion escaping as ammonia gas into the surrounding air. The filtrate was adjusted to 100mL with nano pure water.

Ammonia Determination by Ion Selective Electrode (ISE)

The level of ammonia in the sample was determined using an ammonia selective electrode (Orion model 9512HPBNWP from ThermoScientific). The ammonia selective electrode was set up, maintained and stored according to the user guide manual provided

by the manufacturer. It was connected to a model 420A Orion pH meter, and the pH meter was set to read in milli-volts (mV).

A series of ammonia standard solutions (1, 10, and 50ppm) was prepared by diluting a 1000ppm commercial ammonia standard solution (Ricca Chemical Company) into a solvent. 100mL of each standard solution was freshly prepared daily and used to construct a standard curve. A standard curve was constructed by plotting the milli-volt (mV) readings against the logarithm of ammonia concentration. The levels of ammonia in the exposed meat samples were determined by using a regression equation obtained from the standard curve.

A 50mL aliquot of each of the 100mL standards (1, 10, and 50ppm) or sample solutions was transferred into a 100mL beaker. Measurement of ammonia in solutions using the ISE was conducted according to the ISE user guide by adjusting the ionic strength and pH of the extract with pH-adjusting ISA (Ionic Strength Adjusting) solution from ThermoScientific. As soon as the electrode was placed into the 50mL standard solution or filtrate, the pH of the solution was brought to above 11 by adding 1.6mL of pH-adjusting ISA and was mixed evenly with a magnetic stir bar at constant moderate speed. The mV was recorded once it was stable. Measurements were made in order starting with standards (low to high), control and samples.

Statistical Analysis

The data was analyzed using the SAS program (Version 9.2, SAS Inst. Inc., Cary, N.C., U.S.A., 2002). PROC MIXED was used to determine the significant differences among control, exposed, and treated meat samples.

Results and Discussion

Exposing meat samples to 200ppm NH_3 in N_2 gas for 4 hours resulted in ammonia contamination ranging from 220 to 300ppm. Based on Figure 18, the ammonia concentration in meat samples which had been through an ammonia removal treatment showed no significant differences ($P<0.05$) from the ammonia content of exposed meat samples. All of the removal methods (1 hour and 2 hours air flushing, vacuum treatment and 2%acetic acid rinsing) showed minimal ammonia reduction in the meat samples. The percentage of reduction for all the removal methods was $<10\%$ as shown in Figure 19, although the 2%acetic acid rinsing showed the highest %reduction of ammonia concentration which was $8.3\pm4.4\%$. It also lowered the pH of the exposed meat from 5.83 ± 0.05 to 5.57 ± 0.05 after acid rinsing. Secondly, the vacuum treatment had a %reduction of $6.6\pm3.8\%$. Those two methods were possibly only effective in reducing the amount of ammonia present on the surface of the contaminated meat sample. The ammonia which had already diffused into the meat matrix was not effectively removed by those methods. Anil (1971) found that the first layer of 0.6cm (1/4inch) beef muscle exposed to ammonia contained the highest amount of ammonia contamination. The ammonia concentration in third muscle layer of 0.6cm (1/4inch) was the same as the ammonia concentration in the third layer of the control meat sample (Anil, 1971).

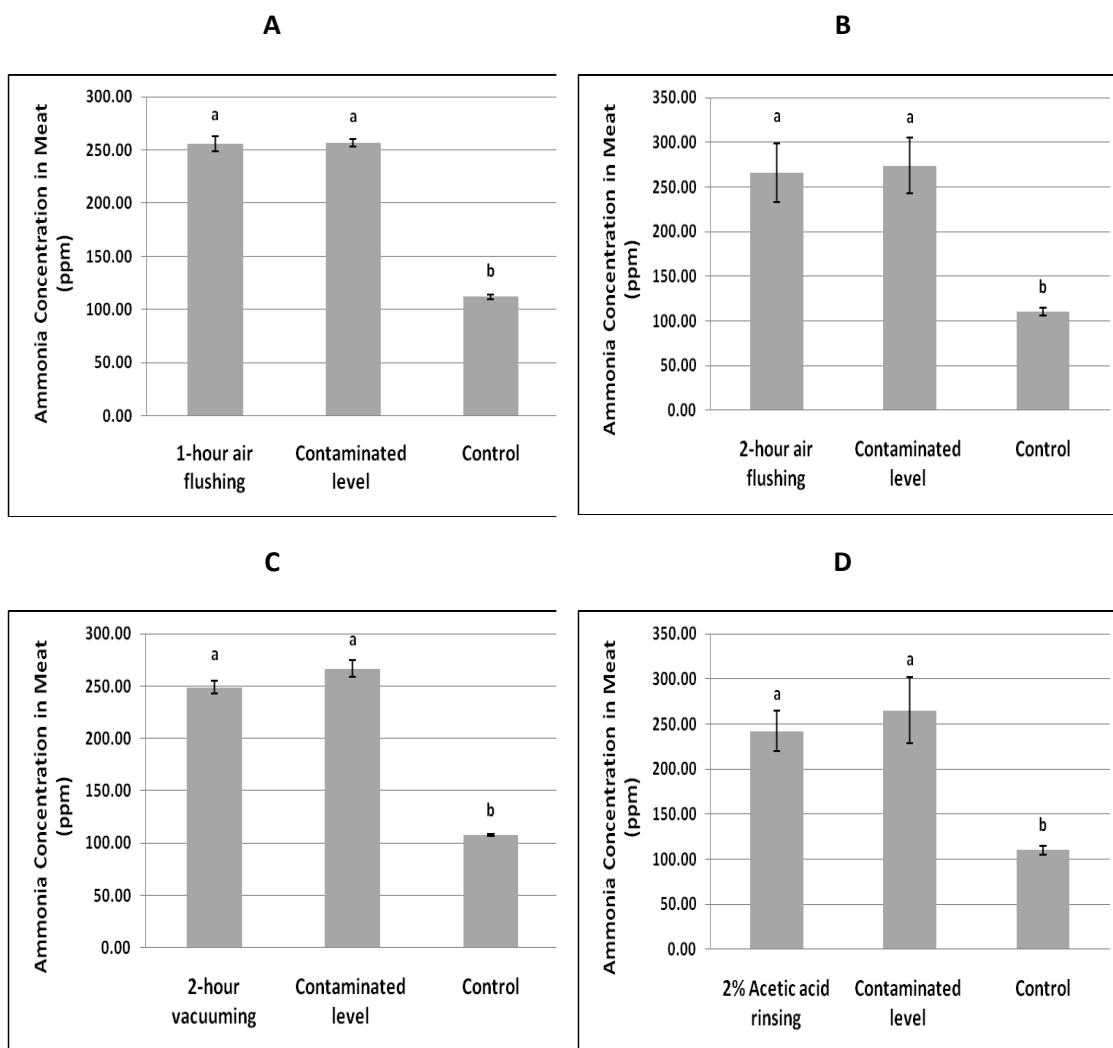


Figure 18. Ammonia concentrations (ppm) in meat samples. A: 1-hour air flushing, contaminated level, and control; B: 2-hour air flushing, contaminated level, and control; C: Vacuum treatment, contaminated level, and control; D: 2%acetic acid rinsing, contaminated level, and control. The concentrations of ammonia in each treatment plotted are means \pm standard deviation from 3 replications. Means with different superscript letters in each treatment are significantly different at $P < 0.05$. Ammonia concentrations plotted are ammonia concentration (ppm) without subtracting the ammonia background (control)

Al-Sahal (2003) air-flushed ammonia contaminated meat samples for 30 and 60 minutes at room temperature, and he observed a reduction in pH of the meat sample's surface in comparison to the same ammonia contaminated meat sample's surface without aeration. Since aeration could reduce the pH in ammonia contaminated meat samples, it

could also reduce ammonia concentration in contaminated meat (Al-Sahal, 2003). However, based on our results (Figure 18), reduction of ammonia by flushing meat samples with clean air was not effective. The ammonia contamination level in air-flushed samples was only reduced by approximately 1.4 ± 8.4 and 7.9 ± 4.0 ppm from the total contamination in exposed meat for 1 hour and 2 hours flushing, respectively. Reduction of surface pH observed after aeration of contaminated samples by Al-Sahal (2003) might be caused by the interaction between CO_2 in surrounding air and the meat surface, resulting in lower pH on the meat surface. Thus, a decrease in pH was not necessarily an indication of reduction of ammonia concentration in contaminated meat samples.

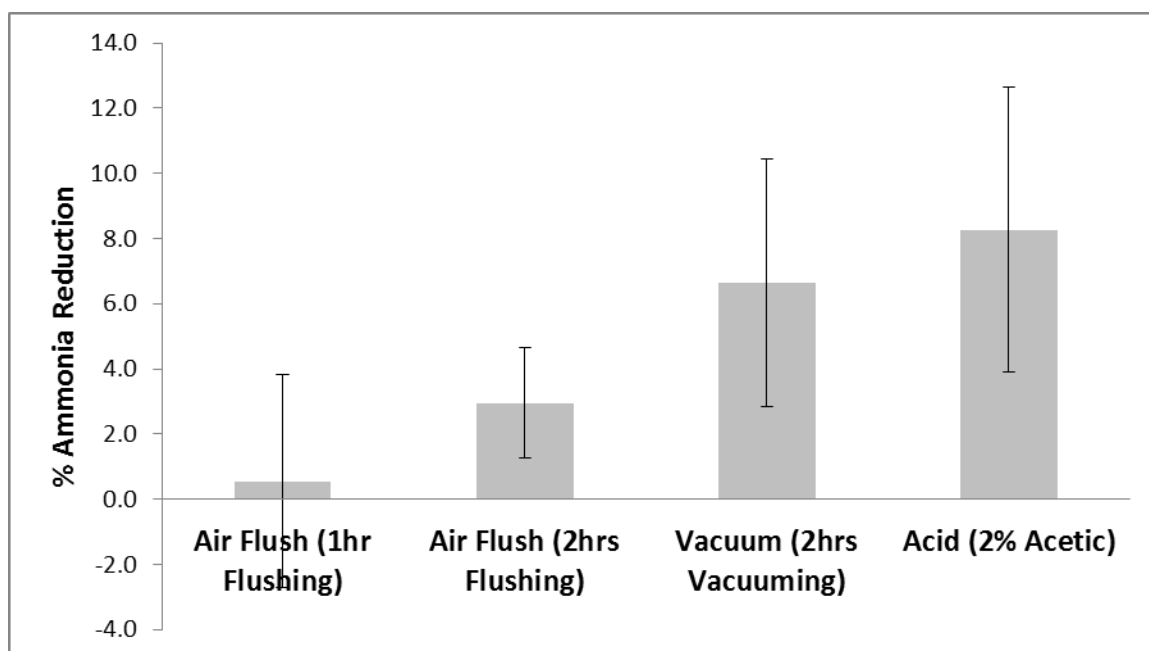


Figure 19. Percent ammonia reduction by each removal treatment - 1-hour air flushing, 2-hour air flushing, vacuum treatment, and 2% acetic acid rinsing.

Conclusion

Removal of ammonia in contaminated meat by flushing with clean air, vacuum treating, and acetic acid rinsing was not effective in reducing ammonia contamination. Both vacuum treatment and rinsing contaminated meat samples with 2% acetic acid might be slightly more effective than air flushing to reduce ammonia concentration on the meat surface. Rinsing with acetic acid was found to lower the pH of the meat. A decrease in pH during aeration was not an indication of effectiveness of ammonia reduction. A possible way to reduce ammonia contamination effectively in exposed meat may be by trimming the surface of the contaminated meat by 0.6cm (1/4inch), and then applying either vacuum treatment, acid rinsing or air flushing.

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SUMMARY

The ion selective electrode method could be an effective method for on-site ammonia screening. The extraction procedure plays an important role in obtaining good recovery and reproducibility results for the ion selective electrode method. Our study concluded that blending after grinding the meat muscle was an effective way to extract ammonia out of the meat matrix. Using pH 6 0.01M potassium phosphate buffer (PPB) as the solvent helps to maintain the pH of the solvent and reduce the rate of ammonia lost to the surrounding air during the extraction process, especially filtration. By combining all the steps (grinding, blending, centrifuging, and filtering) and using pH 6 0.01M PPB, our results showed recoveries >90% with coefficients of variation ranging from 3.6 to 14.2% for ammonia concentrations ranging from 10 to 200ppm in spiked meat samples.

Having a high water content and low pH, meat is very susceptible to ammonia contamination. The rates of ammonia uptake in fresh meat exposed to 200ppm NH_3 gas at 20-25°C and 3-5°C were more rapid than the rate of ammonia uptake in frozen meat exposed to the same concentration of NH_3 gas at -13°C. The state of the meat samples (frozen vs. fresh) affects the rate of ammonia uptake. Fresh meat absorbed ammonia from the surrounding air with rates of 58.4 ± 7.1 ppm per hour for 20-25°C during a 6-hour exposure, and 56.4 ± 5.8 ppm per hour for 3-5°C during a 9-hour exposure. Saturation began to occur when exposing fresh meat for more than 9hours at 3-5°C. Meat has the ability to absorb and accumulate ammonia in its system. Our results showed that exposing fresh meat to 200ppm NH_3 gas for 12hours resulted in 586.7 ± 14.2 ppm ammonia in meat; thus, longer exposure times could lead to high concentrations of ammonia in exposed meat which exceed the concentration of NH_3 to which the meat was

exposed. Moreover, our result showed that Cryovac B6620 packaging film has the ability to protect meat from ammonia contamination.

Treating contaminated meat by flushing with clean air, vacuum treating, and acetic acid rinsing was not effective in reducing ammonia contamination. The percentage of ammonia reduction for all the removal methods was <10%. Both vacuum treatment and rinsing contaminated meat samples with 2% acetic acid might have the ability to reduce the amount of ammonia present on the surface of the contaminated meat sample. Ammonia diffused into the meat matrix was not effectively removed by those methods.

APPENDIX

Spiking Level (uL)	Ammonia Concentration in Meat (ppm)	Average Recovery of Ammonia (%)	%CV
100	10	95.3 \pm 3.6	3.7
200	20	97.8 \pm 6.0	6.1
500	50	94.5 \pm 3.3	3.5
1000	100	95.3 \pm 4.1	4.3
2000	200	96.1 \pm 3.5	3.6

Table A-1. Recovery (%) and coefficient of variation (%CV) of ammonia in spiked meat samples (n=3) extracted by blending meat with pH 6 of 0.02M potassium phosphate buffer (PPB) and measured by ammonia selective electrode (ISE). A set of standards (1, 10, and 25ppm) diluted with pH 6 of 0.02M PPB was used to construct the standard curve. The recovery of ammonia in each sample was calculated by subtracting the ammonia background (control) from the total ammonia concentration in the sample.

Spiking Level (uL)	Solvents					
	Nano Pure Water		pH 6 of Nano Pure Water		pH 6 0.02M Potassium Phosphate Buffer	
	Average Recovery of Ammonia (%)	%CV	Average Recovery of Ammonia (%)	%CV	Average Recovery of Ammonia (%)	%CV
100	124.1 \pm 4.3	3.5	177.8 \pm 3.2	1.8	113.0 \pm 2.8	2.5
200	81.6 \pm 8.3	10.2	111.2 \pm 2.2	2.0	71.8 \pm 0.5	0.7
500	61.9 \pm 4.1	6.6	81.2 \pm 2.7	3.3	56.2 \pm 1.3	2.3
1000	79.1 \pm 11.3	14.3	84.2 \pm 0.1	0.1	70.7 \pm 6.4	9.0
2000	120.9 \pm 1.3	1.1	100.0 \pm 0.0	0.0	105.2 \pm 6.2	5.9

Table A-2. Average recovery (%) of ammonia in spiked meat samples (n=2) extracted by blending meat with different solvents and measured by ammonia selective electrode. A set of spiked meat extracts was used as standards to construct the standard curve. The standards were prepared the same way as the samples. The recovery of ammonia in each sample was calculated by subtracting the ammonia background (control) from the total ammonia concentration in the sample.

Spiking Level (μL)	Solvents					
	Nano Pure Water		pH 6 of Nano Pure Water		pH 6 0.02M Potassium Phosphate Buffer	
	Average Recovery of Ammonia (%)	%CV	Average Recovery of Ammonia (%)	%CV	Average Recovery of Ammonia (%)	%CV
100	154.3 ± 150.4	97.5	129.3 ± 7.8	6.0	101.6 ± 58.9	57.9
200	140.4 ± 45.4	32.3	122.5 ± 17.4	14.2	125.9 ± 11.6	9.2
500	115.3 ± 11.5	10.0	93.2 ± 0.4	0.4	113.1 ± 1.4	1.2
1000	107.7 ± 3.1	2.9	94.1 ± 5.5	5.8	119.5 ± 3.6	3.0
2000	102.6 ± 1.7	1.7	100.1 ± 2.0	2.0	111.1 ± 9.4	8.5

Table A-3. Average recovery (%) of ammonia in spiked meat samples (n=2) extracted by blending meat with different solvents and measured by cation-exchange chromatography (IC). A set of spiked meat extracts was used as standards to construct the standard curve. The standards were prepared the same way as the samples. The recovery of ammonia in each sample was calculated by subtracting the ammonia background (control) from the total ammonia concentration in the sample.

Gauge	8" – 15" Widths		16" – 24" Widths	
Shrink Temperature, Water °F	185-195		185-195	
Haze (%)	4.4		6.0	
Gloss (%)	81		78	
Instrumented Impact: Peak Load (N)	188		200	
Water Vapor Transmission Rate [g/(100 in ² -24 hrs.)]@100% RH, 100 °F	0.4-0.5			
Oxygen Transmission Rate (1cm ³ (STP)/(m ² -24 hr-atm))@0%RH, 40 °F	3-6			
Carbon Dioxide Transmission Rate (cm ³ (STP)/(m ² -24 hr-atm))@0%RH	66			
	LD*	TD**	LD*	TD**
Tensile Strength (psi @ 73°F)	11,100	11,000	10,600	11,400
Elongation at Break (%)	133	157	140	150
Unrestrained Shrink @ 185°F (%)	25	40	25	35
Unrestrained Shrink @ 195°F (%)	36	46	35	42

Table A-4. The properties of Cryovac Type B6620 film (by Sealed Air Corporation, Duncan, SC). *Longitudinal Direction. ** Transverse Direction